Water Resources Research Institute of the University of North Carolina Annual Technical Report FY 2007

Introduction

During the Fiscal Year 2007 reporting period, the Water Resources Research Institute of The University of North Carolina supported research from 8 universities for 26 related projects. Research priorities, as directed by the WRRI (Water Resources Research Institute) Advisory Committee included the following:

A. Water Availability, Use and Climate Interaction 1. Water use: Research is needed to improve processes for collecting data and estimating use of water in North Carolina. 2. Effectiveness of conservation measures: Research is needed to improve estimates of the effectiveness of conservation measures for urban water use during droughts. 3. Climate forecast and water availability during droughts: Incorporate the climate forecast to improve the ability to manage reservoirs. Determine frequency, duration, and recovery from periods of low precipitation.

B. Drinking Water Quality 1. Contaminant screening: Evaluate current and new techniques to screen drinking water regulated contaminants and other contaminants of special health concern at water intakes and upstream sampling locations. 2. Compliance with drinking water standards: Evaluate how water suppliers can most effectively comply with regulations for disinfection by−products and other difficult−to−comply parameters. 3. Groundwater quality and safety: Evaluate criteria for groundwater standards, groundwater aquifer storage and recovery systems; groundwater safety for individual homes and small public systems with special attention to arsenic and radon. 4. Evaluate and monitor lead, copper and other metals in household plumbing. 5. Water supply security: Determine effective approaches/strategies to protect water supplies from the source to the end user.

C. Nutrients and Water Quality 1. Chlorophyll á standard: Determine effectiveness of the chlorophyll á standard as a water quality indicator. 2. Nutrient balance in watersheds: Determine how various watershed nutrient sources and sinks respond to changes over time. Determine the expected time lags in changes to surface and ground water nutrient loads. Determine the hydrological influences on nutrient cycling. Evaluate the different approaches/strategies to water monitoring and the costs involved.

D. Urbanization Impacts on Water Quality 1. Stormwater Management: Evaluate how well stormwater best management practices (BMPs) and management strategies protect downstream water quantity and quality. Determine which practices are the most effective for addressing coastal stormwater discharges. Estimate the monetary benefit of the BMPs and improvements. 2. Low Impact Development (LID): Determine which LID techniques work best in North Carolina to minimize development impacts to watersheds.

E. Monitoring and Assessment of Special Management Programs Determine the dynamics of fecal coliform, sediment and nutrients in urban streams and stormwater detention facilities in the Jordan Lake Reservoir watershed, Neuse River Basin, Tar−Pamlico River Basin, and other areas that are subject to TMDLs.

F. Agricultural Impacts 1. Research is needed to determine water quality impacts (nutrients and fecal coliform) of special classes of agricultural operations such as land application of animal waste, pasture operations, municipal biosolids, and organic farming. 2. Determine whether there is a nitrogen reduction benefit in partial lagoon covers and re−use methane systems. Determine the effect of spray operations on nitrogen runoff.

G. Aquatic Ecosystem Functions and Instream Flow Needs There are a variety of ecosystem function issues that need to be researched and evaluated to improve water quality and habitat protection: 1. Effectiveness of riparian buffers in restoration, especially in the mountain region. 2. Effective approaches to aquatic ecosystem restoration 3. Strategies and effectiveness of aquatic weed control 4. Instream flow and wetland functional assessment approaches 5. Degradation of trout streams 6. Protection of rare and endangered aquatic species in

North Carolina streams.

H. Waste Management 1. Septic systems: Evaluate the contributions of septic tanks to groundwater and nearby streams. 2. Biosolids disposal: Determine fate and transport of nutrients and pathogens from agricultural uses of biosolids. Determine alternative uses and markets for biosolids generated from wastewater.

I. Water Quality Monitoring 1. Mercury: Research is needed to better understand sources and transport mechanisms for mercury in water, sediment, and tissue. A statewide assessment of conditions that favor methyl mercury is needed. 2. Effectiveness of water quality monitoring across North Carolina: More effective monitoring is needed for permitting, modeling use support, and assessment of trends. Determine frequency of sampling in relation to environmental indicators and nutrient interactions. 3. Economic and feasibility assessment of water quality and remote environmental monitoring programs 4. Biological stressor studies in impaired waters

J. Groundwater 1. Shallow groundwater of the Coastal Plain: Determine the relationship between the Coastal Plain's surficial aquifers, surface water, and the first confined aquifer. Determine the relationship between the transfer of contaminants and waste discharges from land and surface waters to surficial aquifers and vice versa. 2. Naturally occurring chemicals: Compile latitude and longitude coordinates and well and water depth of areas that have naturally occurring chemicals in soil that affect groundwater. Working with USGS datasets would be useful.

Research Program Introduction

The Water Resources Research Institute of The University of North Carolina is responsible for fostering and developing a research training and information dissemination program responsive to the water problems of the State and region. To develop its programs, the Institute maintains an aggressive effort to interact and communicate with federal, state, and local water managers. The close contact with water managers is a basis for determining the ever−changing water research priorities.

Priority water research needs for the FY 2007 program were developed in close consultation with the Institutes' Advisory Committee. Following their annual meeting, a statement of priority research needs was developed. The proposal solicitation, as in the past, is sent to all presidents and relevant department heads of senior colleges and universities in North Carolina as well as historically black colleges, to apprise them of the opportunity to submit proposals. The call for proposals is also sent to an email distribution list of approximately 180 university faculty across North Carolina. The proposals received are sent to the Technical Committee and to external peer reviewers to determine the relevancy, need for the proposed research and relative strength and weaknesses. The Technical Committee meets to review all comments made by reviewers and make recommendations regarding proposal funding. Factors considered in the review of proposals are: (1) scientific quality of the proposed work; (2) need for the results of the research in North Carolina and the region; (3) the probability that useful results can be obtained in one−year; (4) the cost of the proposed work; (5) opportunities for application in teaching; (6) the usefulness of the results for managing water resources in North Carolina, and (7) how closely the project relates to the WRRI mission.

Endocrine and Reproductive Effects of the Pharmaceutical Fluoxetine on Native Freshwater Mussels: Proximity to Measured Environmental Concentrations

Basic Information

Title:	Endocrine and Reproductive Effects of the Pharmaceutical Fluoxetine on Native Freshwater Mussels: Proximity to Measured Environmental Concentrations
Project Number:	2006NC61B
Start Date:	3/1/2006
End Date:	8/31/2007
Funding Source:	104B
Congressional District:	2
Research Category:	Water Quality
Focus Category:	Toxic Substances, Water Quality, Surface Water
Descriptors:	Unionid Mussel, endocrine disruptor, pharmaceutical, toxic substances, pollutants
Principal Investigators:	W. Gregory Cope, Robert B Bringolf, Rebecca M Heltsley, Damian Shea

Publication

1. Bringolf, R. B., R. M. Heltsley, C. Eads, T. J. Newton, S. Fraley, D. Shea, and W. G. Cope. 2007. Effects of fluoxetine on freshwater mussel reproduction: relation to environmental occurrence. Annual Meeting of the Water Resources Research Institute of North Carolina, Raleigh, NC, March 27−28, 2007
2. Bringolf, R. B., R. M. Heltsley, C. Eads, T. J. Newton, S. Fraley, D. Shea, and W. G. Cope. 2007. Environmental occurrence of fluoxetine and its effects on freshwater mussel reproduction. 5th Biennial Symposium of the Freshwater Mollusk Conservation Society, Little Rock, AR, March 12−15, 2007.
3. Heltsley, R. M., W. G. Cope, R. B. Bringolf, C. B. Eads, and D. Shea. 2006. Environmental concentrations of prozac induce spawning in freshwater mussels. 27th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Montreal, Canada, November 5−9, 2006.
4. Heltsley, R. M., W. G. Cope, R. B. Bringolf, C. B. Eads, and D. Shea. 2006. Prozac elicits spawning in native freshwater mussels. 232nd Annual Meeting of the American Chemical Society, San Francisco, CA, September 10−14, 2006.
5. Cope,W. Gregory; Damian Shea, Robert Bringolf, Rebecca M. Heltsley,2007,WRRI−382, Endocrine and Reproductive Effects of The Pharmaceutical Fluoxetine on Native Freshwater Mussels: Proximity to Measured Environmental Concentrations, NC Water Resources Research Institute, NC State University, Raleigh, NC, 27.

ENDOCRINE AND REPRODUCTIVE EFFECTS OF THE PHARMACEUTICAL FLUOXETINE ON NATIVE FRESHWATER MUSSELS: PROXIMITY TO MEASURED ENVIRONMENTAL CONCENTRATIONS

ABSTRACT

Fluoxetine, a selective serotonin reuptake inhibitor (SSRI) drug commonly prescribed as an antidepressant, is increasingly being detected in surface waters impacted by treated municipal wastewater. Because of its serotonergic action, fluoxetine (and other SSRIs) has been used to manipulate reproductive processes in mollusks. We evaluated the effects of acute water-only fluoxetine exposure on reproductive activities, including release of gametes, parturition of glochidia (larvae), and mantle lure display behavior in native freshwater mussels (Family Unionidae). In *Elliptio complanata*, 3000 μg/L of fluoxetine significantly induced release of spermatozeugmata in male mussels during a 48 h exposure. Fluoxetine significantly induced parturition of nonviable glochidia from adult female *E. complanata* exposed to 300 ($p = 0.0118$) and 3000 μg/L ($p < 0.0001$) when compared to controls. Fluoxetine exposure also resulted in stimulation of mantle flap display behavior in adult female *Lampsilis fasciola* and *Lampsilis cardium*. Mussels exposed to 300 μg/L ($p = 0.0075$) and 3000 μg/L ($p = 0.0001$) of fluoxetine were more frequently observed in the most advanced stages of display (stages 5 and 6) compared to control mussels. Accordingly, mussels in the 300 μg/L ($p = 0.0341$) and 3000 μg/L ($p = 0.0006$) fluoxetine treatments were less frequently observed in less advanced display stages (stages 1 & 2) compared to control mussels. Although the lowest observed effects concentrations for fluoxetine in these tests (300 μg/L) are greater than concentrations measured in surface water, they demonstrate that fluoxetine exposure has the potential to disrupt several aspects of reproduction in freshwater mussels native to the United States, a faunal group recognized as one of the most imperiled in the world. Additional research is needed to elucidate the effects of chronic, low-level exposure to fluoxetine and other drugs with the same mechanism of action.

INTRODUCTION

Pharmaceuticals and personal care products (PPCPs) have been detected in surface waters, effluent from municipal wastewater treatment facilities, seawater, and groundwater (for a review, see Fent et al., 2006). Although these compounds are generally not as persistent as traditional priority pollutants (e.g., polychlorinated biphenyls and organochlorine pesticides), the continuous release of PPCPs into our rivers and streams presents similar exposure conditions as that of a persistent organic pollutant (Johnson et al., 2005). Previous studies indicate that many of these compounds enter the environment in their parent form (un-metabolized) or as a mixture of metabolites (Daughton and Ternes, 1999; Johnson et al., 2005; Kolpin et al., 2002; Metcalfe et al., 2003; Vieno et al., 2007). In addition, some PPCPs have been found to accumulate in fish in effluent-dominated streams (Brooks et al., 2005). Therefore, compounds that were manufactured with the intent of being bioactive are being discharged into surface and ground waters and may be responsible for acute and chronic effects in aquatic organisms including endocrine disruption (Colborn et al., 1993; Routledge et al., 1998).

Native freshwater mussels (family Unionidae) are long-lived (30-100 yr) filter- and deposit-feeding benthic organisms that live burrowed in sediments of streams and rivers. They are among the most rapidly declining faunal groups in North America; approximately 67% of the nearly 300 freshwater mussel species found in North America are considered vulnerable to extinction or already extinct (Bogan, 1993; Williams et al., 1993). The decline of mussel populations in North America has occurred steadily since the mid 1800s and has been attributed to pollution, construction of dams and impoundments, sedimentation, navigation, and habitat degradation (Bogan, 1993; Brim Box and Mossa, 1999; Neves, 1997; Vaughn and Taylor, 1999). Principally, the stressors associated with human development and urbanization have hastened these population declines over the past 20 to 50 years.

Unionids have a unique life history that includes an obligate parasitic larval stage (glochidia). Gravid female mussels release glochidia which must successfully attach to gills or fins of a suitable host fish. Mussels of the tribe Lampsilini use elaborate and highly specialized mantle tissue displays to mimic prey items and attract potential host fish (Haag et al., 1995; Haag and Warren, 1999). As the fish strikes the mantle flap "lure," the gill epithelial tissue is ruptured and hundreds to thousands of glochidia are released into the buccal cavity of the fish where some attach to gills and undergo transformation into juvenile mussels before releasing from the fish and settling into the sediment to become adults. Because of this unique life history, unionid mussels may be among the groups of aquatic organisms most susceptible to PCPPs and other endocrine disrupting compounds (EDCs) that are now found in our surface waters.

One of the PPCPs detected frequently in surface waters is fluoxetine, the active ingredient in Prozac® and other anti-depressant drugs (Kolpin et al., 2002; Metcalfe et al., 2003; Ternes, 1998). Fluoxetine is a selective serotonin reuptake inhibitor (SSRIs) drug and exerts its action by reducing the clearance rate of serotonin, (5-hydroxytrypamine) a neurotransmitter, in the synapse following nerve signal transmission.

Serotonin is a key mediator for a wide variety of physiological functions in mollusks. In bivalve mollusks serotonin regulates a reproductive processes including oocyte maturation (Fong et al., 1994; Hirai et al., 1988) spawning (Hirai et al., 1988; Ram et al., 1993) and parturition (release of glochidia; Fong et al., 1996). Serotonin, fluoxetine and other SSRIs have been used to artificially induce spawning in freshwater bivalves for aquaculture purposes (Cunha and Machado, 2001; Gibbons and Castagna, 1984) and have been investigated as a potential chemical control mechanism (i.e., disruptor of reproduction) for exotic bivalve species like the zebra mussel *Dreissena polymorpha* (Fong et al., 1994; Fong et al., 1996; Ram et al., 1992). In light of the increasing number of surface waters with measurable fluoxetine (and other SSRI) concentrations and the imperiled status of freshwater mussel fauna, determination of the effects of environmental exposures of fluoxetine to native freshwater mussels is of utmost importance.

The goal of the present study was to resolve the effects of fluoxetine on reproduction in native freshwater mussels. Specific objectives were to determine 1) the concentration of fluoxetine required to induce parturition of glochidia from brooding female mussels, 2) if fluoxetine alters mantle flap display behavior used to attract a fish host, 3) and if fluoxetine exposure induces spawning from male mussels.

MATERIALS AND METHODS

Test organisms

Adult *Elliptio complanata* mussels were collected from the Eno River near Hillsborough, North Carolina and Little Creek near Wilson, North Carolina, in July 2004, June 2005, April 2006 and July 2006. These are relatively uncontaminated, rural, forested streams in the central Piedmont of North Carolina. The mussels were transported using methods of Cope et al. (2003) to the Aquatic Toxicology Laboratory at North Carolina State University. Adult female *Lampsilis cardium* (length 93 to 123 mm) displaying mantle flap lures were collected from Pool 8 of the Upper Mississippi River near La Crosse, Wisconsin, in September 2005 and shipped to the Aquatic Toxicology Laboratory at North Carolina State University via overnight courier following methods described by Cope et al. (2003). Adult female *Lampsilis fasciola* (length 45 to 82 mm) displaying mantle flap lures were collected from a rural, largely forested reach of the Little Tennessee River near Franklin, North Carolina, in May 2006 and transported to the Aquatic Toxicology Laboratory at North Carolina State University (Cope et al., 2003). Upon arrival at the laboratory, all mussels were acclimated to reconstituted soft water (ASTM, 1993) over 24 h and maintained at 18-20°C for at least 24 h prior to beginning any experiments to ensure that spawning or release of glochidia during experiments was not a result of handling or transport stress.

Test Chemicals

Fluoxetine hydrochloride (Sigma-Aldrich, St. Louis, Missouri, USA; 98% purity) and serotonin creatinine sulfate (Acros Organics, Geel, Belgium; 99% purity) were purchased from VWR International (West Chester, PA, USA). Working solutions of fluoxetine (8 mg/mL and 0.008 mg/mL) were prepared in deionized water to a final volume of 10 mL. The working solution of serotonin (20 mg/mL) was also prepared in deionized water. Test containers were pre-conditioned with appropriate test solutions for 24 h before experiments began.

Water Chemistry

Test solutions were prepared in reconstituted soft water for all experiments. Water chemistry (temperature, pH, dissolved oxygen, conductivity, alkalinity, and hardness) was measured according to standard methods (APHA, 1995) in one replicate of each treatment at least at the beginning and end of each test. A calibrated multiprobe (YSI Model 556 MPS, Yellow Springs Instruments, Yellow Springs, OH, USA) was used for analysis of pH, dissolved oxygen, conductivity and temperature. Alkalinity was determined by titration with 0.02 N H_2SO_4 to pH 4.5, and hardness by titration with 0.01 M ethylenediaminetetraacetic acid (EDTA).

Glochidia Parturition in *Elliptio complanata*

Glochidia release by brooding female *E. complanata* mussels (length from 55 to 77 mm) following exposure to fluoxetine was determined in three trials (July 2004, June 2005, and July 2006).

Female mussels that had not released glochidia during acclimation were placed in 3.75-L glass aquaria (one mussel per aquaria) containing 2 L of gently aerated reconstituted soft water. The mussels were exposed to one of five fluoxetine treatments (0, 0.3, 3.0, 30, 300, or 3000 μg/L), with 3, 5, or 6 replicates per treatment (depending on the trial) for 96 h. In addition, a serotonin treatment (40 mg/L) was included as a positive control. Mussels were examined at 24 h intervals for the duration of the exposure. Endpoints included time from initiation of exposure to parturition of >100 glochidia and viability of released glochidia, determined by response of a sub-sample (n = 50 -100) of the released glochidia to a saturated solution of NaCl (ASTM, 2006). Composite water samples (10 mL from each replicate in a given treatment) were collected from each treatment for analysis of fluoxetine at time 0 and at the time of first release of glochidia in a replicate.

Mantle Lure Display and Glochidia Parturition in ***Lampsilis cardium*** **and** ***L. fasciola***

Effects of fluoxetine on mantle flap display behavior and glochidia parturition were evaluated in *L. cardium* in September 2005 and in *L. fasciola* in May 2006. Mussels were maintained in 3.75-L glass aquaria (1 mussel/aquaria) containing 2 L of gently aerated reconstituted soft water (ASTM, 1993) and 12 mm of artificial substrate (inert aquarium gravel) at 18-20°C for 24 h prior to beginning the experiment to ensure that the females were displaying their mantle flaps and that spawning or release of glochidia was not a result of handling or transport stress. Only mussels that displayed fish lures and had not released glochidia during acclimation were used for the experiment. The mussels were exposed to one of five fluoxetine treatments (0, 0.3, 3.0, 30, 300, or 3000 μg/L) with 6 replicates per treatment for 96 h in static renewal tests (total of 36 experimental units). All mussels were monitored for mantle flap display and the release of glochidia continuously for the first 6 h of the experiment and then observed at 2 hour intervals during 8 h blocks over the remaining exposure duration. Mantle flap display behaviors were categorized as: Stage 1- shell closed; Stage 2- shell gaped but no mantle tissue exposed; Stage 3- shell gaped and mantle tissue partially extended; Stage 4- shell gaped and mantle tissue fully extended with fish lure visible; Stage 5- shell gaped, fish lure fully extended, and marsupial gills extended beyond shell margin; Stage 6- shell gaped, fish lure fully extended, marsupial gills fully extended, and fish lure pulsating (number of beats/min was quantified). In addition, time to release of glochidia (>100) was recorded and viability was determined. A 100% renewal of exposure water was completed at 24-h intervals to maintain target fluoxetine concentrations. Composite water samples (200 mL from each of 6 replicates) were collected for fluoxetine analysis from each treatment at the time mussels were initially placed in fluoxetine treatments.

Spermatozeugmata Release by ***Elliptio complanata***

Effects of fluoxetine on release of spermatozeugmata or "sperm spheres" (Ishibashi et al., 2000; Waller and Lasee, 1997) were evaluated in male *E. complanata* in April 2006. Because *E. complanata* is not a sexually dimorphic species, we initiated the experiment with 48 non-gravid adult mussels of unknown gender ranging in length from 52 to 74 mm. Mussels were randomly assigned to one of three treatments: control, 300 μg fluoxetine/L, or 3000 μg fluoxetine/L. An experimental unit consisted of a 3.75-L glass aquarium with 1 mussel and 2 L of gently aerated reconstituted soft water (ASTM, 1993).

Temperature was maintained at 20^{o}C throughout the test by partially submerging aquaria in a water bath (Living Stream®, Frigid Units, Toledo, Ohio, USA). Water samples (10 mL) were collected from each aquarium at the start of the experiment (before addition of fluoxetine) and examined at 40x magnification under a dissecting microscope for the presence of spermatozeugmata or glochidia. No mussels had released gametes or glochidia by the start of the experiment. A release of spermatozeugmata was defined as >10 spermatozeugmata per 10-mL sample of exposure water and glochidia parturition was documented when > 100 glochidia were expelled. Water samples were collected and examined at 2-hr intervals for the first 12 h of the experiment and from 24-36 h. A final 10-mL sample was examined at 48 h, after which mussels that had not released sperm or glochidia were exposed to serotonin (80 mg/L) for ≤ 8 h to stimulate release of spermatozeugmata or glochidia they may be carrying. Test solutions were renewed (100%) at 24 h.

Fluoxetine Extraction and Quantification

Exposure water was acidified with formic acid (pH 2.7), filtered (0.45 μm, Whatman, Brentford, Middlesex, UK), spiked with surrogate internal standard, ^{13}C-phenacetin, and extracted using 47 mm C18 Empore™ disks (Varian, Palo Alto, California, USA) that were conditioned according to manufacturer instructions. Following extraction, the disks were dried under vacuum, placed in 50-mL centrifuge tubes, and shaker table extracted with 20 mL of methanol. Methanol was decanted and the extraction was repeated twice more for a total of 60 mL methanol. Extracts were combined and nitrogen evaporated to 4 mL methanol. The extract was then filtered with a UniPrep™ filter (Whatman, Brentford, Middlesex, UK), evaporated to approximately 1 mL, quantitatively transferred to a glass vial and evaporated to dryness. The sample was resuspended in 450 μl of methanol and 50 μl of 2-chlorolepidine was added as a recovery standard.
All analyses were performed with an Agilent 1100 liquid chromatograph coupled to an API 4000 triple-quadrupole mass spectrometer (Applied Biosystems, Foster City, CA). The parent and daughter ions used for each compound were as follows: 13C-phenacetin 181—110, 2-chlorolepidine 179—143, and fluoxetine 310—44 and 310—148. The transitions identified are consistent with previous work on fluoxetine (Brooks et al., 2005). The mass spectrometer was operated in electron spray ionization positive mode. Separation was performed using a Zorbax C18 column (Agilent ZORBAX Eclipse XDB; 250 mm x 5 mm; 5 μm). Solvent A was 10 mM ammonium formate in water (adjusted to pH 2.9 with formic acid). Solvent B was 10 mM ammonium formate and 5 % water in methanol (with an equivalent volume of formic acid). A gradient elution starting with 90 % A ramped linearly to 40 % A over 12 min was used for the separation of the compounds. The system was returned to the initial conditions after 17 min and equilibrated for 6 min. The flow rate was 0.5 mL/min. Samples were quantified using at least a four point calibration curve, which was not forced through the origin. The calibration solutions were not extracted.

Statistical Analyses

For statistical comparisons of glochidia release, the three trials of the experiment in which we evaluated glochidia release in *E. complanata* exposed to fluoxetine were treated as replicates in time (n = 3) and the mean percentage of mussels releasing glochidia in each treatment was determined (total of 21 experimental units). JMP (SAS, Cary, North Carolina, USA) statistical software was used to test for homogeneity of variances (Bartlett's Test) and differences in glochidia release between treatment groups and controls was evaluated with analysis of variance (ANOVA) followed by Dunnett's Test ($\alpha = 0.05$). Display behavior (i.e., frequency of occurrence in each stage) was analyzed by multivariate analysis of variance (MANOVA) followed by Dunnett's Test for means comparison of treatments to controls for each stage (α = 0.05, n = 36 experimental units). For simplicity, frequency of occurrence results were combined in to three categories: stages 1 and 2, stages 3 and 4, and stages 5 and 6. Preceding analysis, frequencies of occurrence in each of the three categories were transformed (arcsine) to achieve homogeneity of variance. JMP (SAS, Cary, North Carolina, USA) statistical software was used for all statistical analyses.

RESULTS

Water Chemistry

Dissolved oxygen concentrations were greater than 80% of saturation at all times during all experiments. Other water quality parameters were consistent among experiments as well: pH ranged from 7.7 to 8.1, alkalinity ranged from 30 to 34 mg $CaCO_3$/L and hardness was 42 to 48 mg $CaCO_3$/L. Mussel survival was 100% in all experiments.

Fluoxetine Analyses

Measured concentrations of fluoxetine ranged from 74.7 to 120.0% of target concentrations for the July 2004 trial and from 85.7 to 111.4% of target concentrations for the June 2005 trial. The most rigorous sampling for determination of fluoxetine in laboratory exposure water was during the July 2006 trial in which samples were collected at time 0, 12 h, 24 h and 48 h. Measured fluoxetine concentrations ranged from 78 to 169% of target concentrations throughout the experiment (Table 1). Surrogate (2-chlorolepidine) recovery ranged from 80 to 100 % for all *E. complanata* experiments.

Table 1. Measured fluoxetine concentrations in composite water samples (from 6 replicates) from laboratory *Elliptio complanata* exposures during June 2005; these values are similar to measured concentrations from the *E. complanata* experiment in July 2006.

Target fluoxetine concentration (µg/l)	Measured fluoxetine concentration (µg/l)					
	Time (hour)					
	0	12	24	48	Mean	SD[a]
Control	BDL[b]	BDL	BDL	BDL	--	--
0.3	0.5	0.26	0.29	0.20	0.3	0.1
3	3.8	2.4	1.2	2.5	2.5	1.1
30	36.8	15.8	15.4	23.2	22.8	10.0
300	331.3	243	190.9	283.5	262.2	59.7
3000	2340.5	2597.0	1629.7	NA[c]	2189.0	501.1

[a] Standard deviation
[b] Below detection limit

[c] No samples collected

Glochidia Parturition by *Elliptio complanata*
Fluoxetine induced parturition of nonviable glochidia. A significantly greater percentage of mussels in the 300 μg/L ($p = 0.0118$) and 3000 μg/L ($p < 0.0001$) fluoxetine treatments released nonviable glochidia compared to controls (Figure 1). Mussels in the 0.3 μg/L fluoxetine treatment also released nonviable glochidia; however, this was not significantly different from controls ($p = 0.9998$). One control mussel in the June 2005 trial released nonviable glochidia but no control mussels released nonviable glochidia in the other two trials. Some mussels in each treatment released viable glochidia but there was not a significant difference ($p = 0.8176$) in the percentage of mussels in each treatment that released viable glochidia (Figure 1). Glochidia release by mussels exposed to serotonin, the positive control, was similar to the release by mussels exposed to the highest fluoxetine concentration (Figure 1). Mussels exposed to 300 and 3000 μg/L of fluoxetine and serotonin had marked increase in foot volume, many to the point at which they were unable to close their shell.

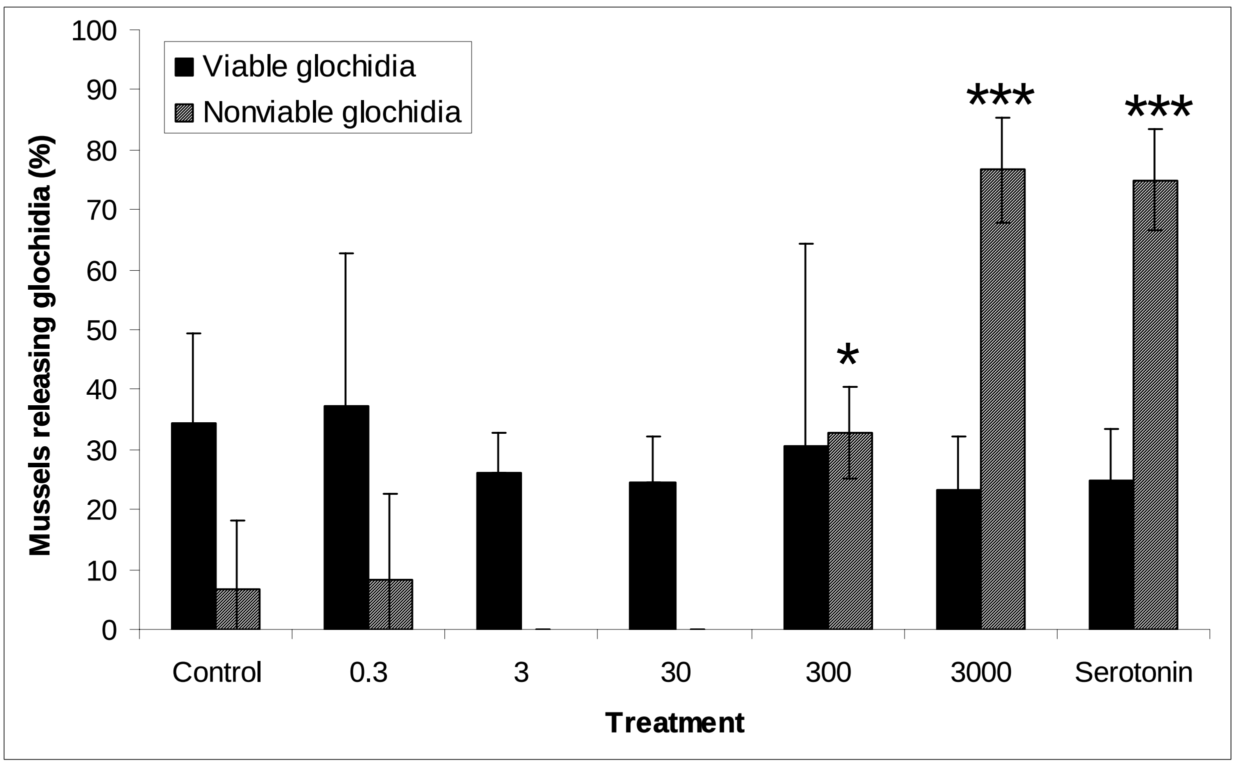

Figure 1. Mean percent of mussels ($N = 14$) per treatment that released glochidia during a 48-h exposure to fluoxetine (0 – 3000 μg/L) or serotonin (40 mg/L). Error bars indicate ± one standard deviation. Asterisks indicate a significant difference compared to control (Dunnett's Test): * $P \leq 0.05$, *** $P \leq 0.0001$.

Mantle Lure Display and Glochidia Parturition
Fluoxetine substantially altered mantle flap display behavior in *L. cardium* and *L. fasciola* in a similar pattern.

In *L. fasciola*, for example, mussels exposed to 300 µg/L (p = 0.0075) and 3000 µg/L (p = 0.0001) of fluoxetine were more frequently observed in the most advanced stages of display (stages 5 and 6) compared to control mussels. Concomitantly, mussels in the 300 µg/L (p = 0.0341) and 3000 µg/L (p = 0.0006) fluoxetine treatments were less frequently observed in less advanced display stages (stages 1 & 2) compared to control mussels (Figure 2). The trend toward more advanced stages of display behavior was more pronounced in mussels exposed to 3000 µg/L of fluoxetine compared to the 300 µg/L treatment group, indicating a concentration-response relationship. Additionally, variability of observation frequency in the various stages among replicates also decreased in the mussels exposed to 3000 µg/L (Figure 2). The results of the experiment with *L. cardium* were consistent with those of *L. fasciola* in terms of a concentration-related response in mantle lure display behavior, except that only mussels in the 3000 µg/L fluoxetine treatment were more frequently observed (p = 0.0001) in the most advanced stages of display (stages 5 and 6) compared to control mussels (data not shown).

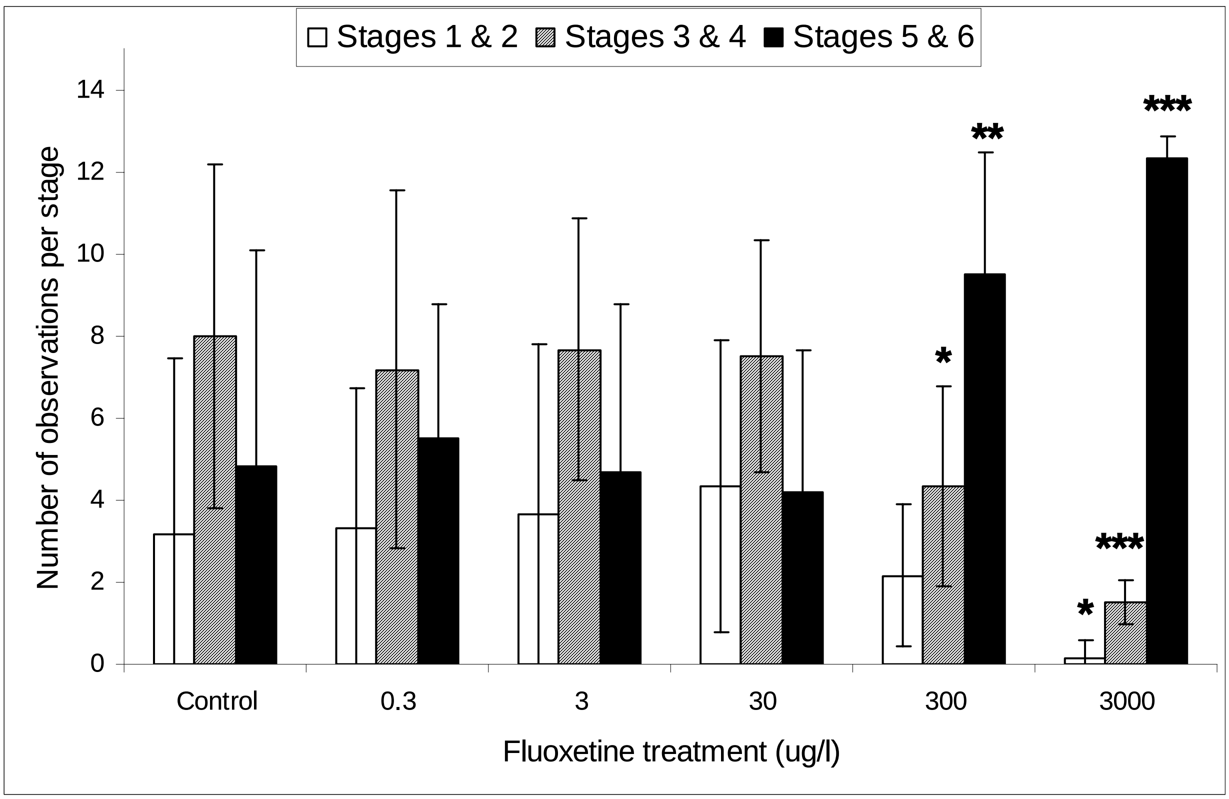

Figure 2. Summary of mantle flap display behavior data for *Lampsilis fasciola* exposed to fluoxetine (0 – 3000 µg/L) for 96 h. Mean number of observations (N = 16 total observations) per display stage (N = 6 replicates). Stages 1 and 2 were no visible mantle flap, stages 3 and 4 were partial displays, and stages 5 and 6 were full displays. Error bars indicate ± one standard deviation. Asterisks indicate a significant difference compared to control (Dunnett's Test): * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

Parturition of glochidia occurred during the mantle lure display experiments with *L. cardium* and *L. fasciola*, as in experiments with *E. complanata*. However, in both of these tests, only mussels in the 3000 μg/L fluoxetine treatment released a significantly ($p < 0.0001$) greater percentage of glochidia compared to controls. All of the glochidia released from mussels during these two experiments were viable glochidia, unlike in the tests with *E. complanata*, in which viable and nonviable glochidia were released. In these tests, the number of glochidia released by mussels exposed to serotonin, the positive control, was again similar to those released by mussels exposed to the highest fluoxetine concentration. Also, as observed in the experiments with *E. complanata*, the foot volume of *L. fasciola* and *L. cardium* was substantially increased in mussels exposed to the two highest fluoxetine concentrations.

Spermatozeugmata Release

Fluoxetine induced release of spermatozeugmata in seven of 16 (43.8%) *E. complanata* of unknown gender in the 3000 μg/L treatment, whereas 1 of 16 (6.3%) mussels in each of the control and 300 μg/L fluoxetine treatments released spermatozeugmata (Figure 3). Release of spermatozeugmata by mussels in the 3000 μg/L fluoxetine treatment was significantly different ($p = 0.0056$) from other treatments. In addition, five of the 16 (31.3%) mussels in the 300 μg/L fluoxetine treatment and 4 of 16 (25.0%) in the 3000 μg/L fluoxetine treatment released glochidia, whereas 2 of 16 (12.5%) released glochidia in the control treatment (Figure 3). Of the 12 remaining mussels that had been exposed to fluoxetine but had not released spermatozeugmata or glochidia within 48 h, 2 released nonviable glochidia within 2 h of exposure to serotonin (80 mg/L). The 10 remaining mussels did not release glochidia or gametes within 24 h of exposure to serotonin.

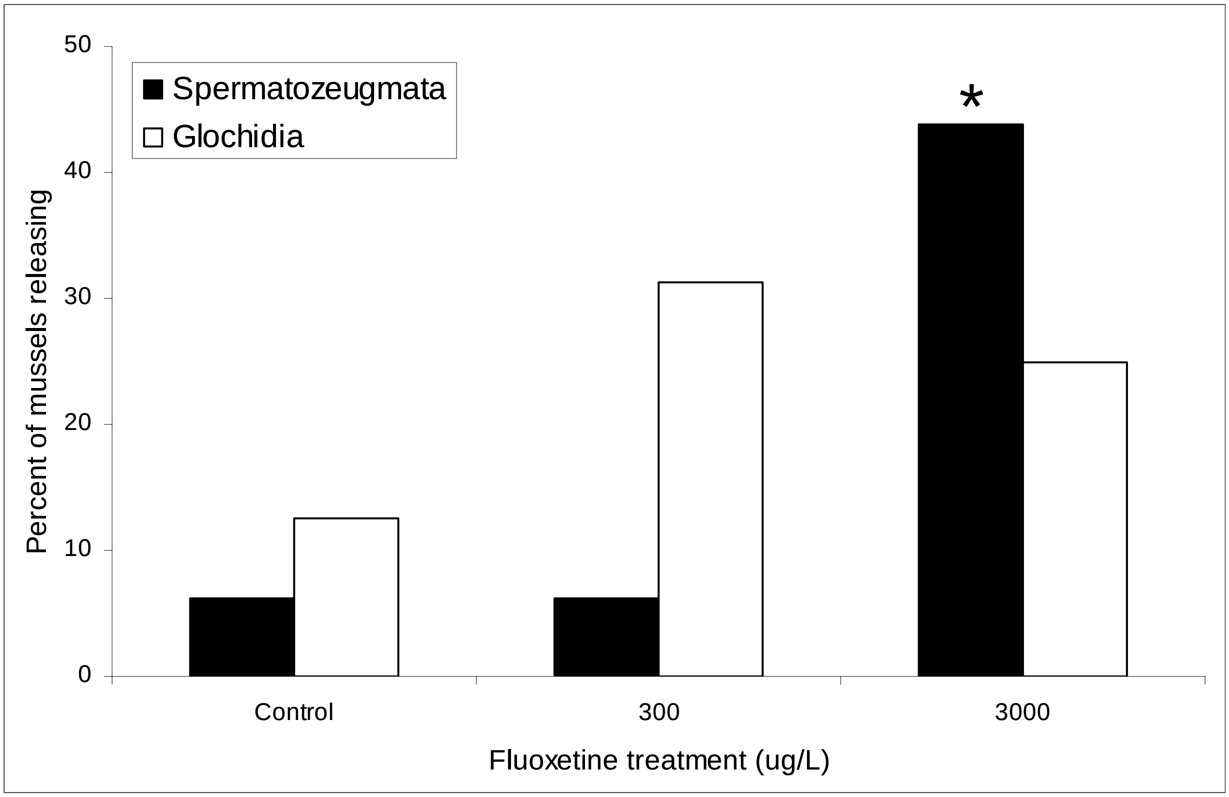

Figure 3. Percent of mussels (N = 16) of unknown gender that released spermatozeugmata or glochidia during 24-h exposure to fluoxetine (0 – 3000 μg/L). Error bars indicate ± one standard deviation. An asterisk (*) indicates a significant difference compared to other treatments (Chi Square; $P \leq 0.05$).

DISCUSSION

The ecological effects of an ill-timed release of larval mussels (glochidia) or gametes caused by environmental fluoxetine exposure could be potentially devastating to localized mussel populations. Likewise, the inability of a female mussel to attract a suitable fish host through altered or ill-timed mantle flap (fish lure) display behavior such that she would not be able to successfully infest a fish with glochidia could also result in reproductive failure and devastate local mussel populations. Our results demonstrate that fluoxetine has the potential to disrupt reproduction of native mussel species by inducing release of spermatozeugmata and glochidia as well as altering mantle flap display behavior. The lowest observed effect concentration (LOEC) of fluoxetine in our study (300 μg/L) is greater than those measured to date in the environment (maximum 0.1 μg/L; Metcalfe, 2003); however, our test durations were relatively short (48 ≤ 96 h) for all experiments. Currently little is known about the effects of chronic, low-level fluoxetine exposure (consistent with surface waters receiving treated wastewater effluent) on mussel reproduction.

Serotonin is a widely occurring biogenic monoamine that regulates a variety of reproductive processes in mollusks including oocyte maturation (Fong et al., 1994), spawning (Fong et al., 1993; Ram et al., 1993; Ram et al., 1996), and parturition (Fong and Warner, 1995) and has been used for enhanced culture of economically important freshwater and marine bivalves. However, high concentrations of serotonin are required to induce spawning and parturition and the concomitant expense can be prohibitive for use in developing countries where aquaculture is becoming increasingly important. As a result, fluoxetine and other SSRI drugs have been investigated as potential alternatives to serotonin and have indeed been reported to induce spawning (Fong, 1998) and parturition (Cunha and Machado, 2001; Fong, 1998) at concentrations that are orders of magnitude lower than required for serotonin and therefore may be more economically feasible than serotonin. The mechanism by which SSRI drugs induce reproductive effects has not been fully elucidated and may involve SSRIs acting as ligands at post-synaptic receptors (acting in a manner similar to serotonin), rather than as inhibitors of reuptake transporters, or may indeed involve blocking reuptake of endogenous serotonin in the post-synaptic cleft.

The LOEC for release of nonviable glochidia by *E. complanata* in the present study was 300 μg fluoxetine/L, but the next lowest test concentration was 30 μg/L, thus the true threshold for effects (in acute tests) lies somewhere between 30 and 300 μg/L. Mussels in all treatment groups released mature glochidia, suggesting that some mussels brought into the lab were on the verge of releasing glochidia in the stream before they were collected and transported to the laboratory. The mussels were collected during June and July when gravid *E. complanata* are observed in North Carolina streams (C. B. Eads, North Carolina State University, Raleigh, NC, personal communication), therefore stress from handling and transport likely stimulated the release of mature glochidia in those individuals. However, the release of immature, nonviable glochidia was related to fluoxetine exposure in a concentration-dependent manner.

Fluoxetine and other SSRI drugs have been used previously to induce spawning and parturition in mussels. Cunha and Machado (2001) used fluoxetine and fluvoxamine, another SSRI drug, to control timing and intensity of glochidia release in *Anodonta cygenea,* for aquaculture purposes. Significant numbers of viable glochidia were released by gravid mussels exposed to 309 and 3090 μg/L of fluoxetine during a 24-h exposure period in their study. *Elliptio complanata* in the present study appeared to be similarly sensitive to fluoxetine (i.e., the LOEC for glochidia release was 300 μg/L). Additionally, consistent with reports by Cunha and Machado (2001) for *A. cygenea*, we observed a strong increase in volume of the foot in *E. complanata* during early stages of exposure to fluoxetine (300 and 3000 μg/L) and serotonin. Cunha and Machado (2001) attributed the increase in foot size to relaxation of foot muscles and the resulting favorable conditions for uptake and storage of water.

To our knowledge this is the first report of reproductive effects with male unionid mussels exposed to an SSRI drug. Male *E. complanata* exposed to 3000 μg fluoxetine/L in the present study released spermatozeugmata (aggregates of hundreds to thousands of spermatozoa) but those exposed to the next lowest concentration, 300 μg/L, did not. Fong (1998) reported induction of spawning in zebra mussels exposed to fluoxetine, fluvoxamine and paroxetine. The lowest concentration of fluoxetine required to induce spawning in zebra mussels was 155 μg/L for males and 1545 μg/L for females in his study. The effects of fluoxetine on viability of spermatozeugmata or individual spermatozoa after release from the sperm sphere have not been elucidated.

In humans, fluoxetine is used for treatment of not only depression, but also for treatment of obsessive –compulsive behaviors associated with Tourette's syndrome (Eapen et al., 1996) as well as convulsive seizures (Pasini et al., 1996), so it is not surprising that it affected mussel behavior in the present study. Consistent with other studies of mollusks in which fluoxetine stimulated reproductive processes (Avila et al., 1996; Fong, 1998; Uhler et al., 2000), fluoxetine exposure resulted in a concentration-dependent stimulation of mantle lure display behaviors by *L. fasciola* and *L. cardium*. It is currently unclear if such behavioral effects would preclude successful attraction of a suitable host fish for glochidia infestation. Additionally, it is not known if fluoxetine (or other SSRIs) would induce display behaviors in female mussels that are not gravid (i.e., out of season), or prematurely induce displays in those that contain immature or nonviable glochidia. Likewise, additional research is required to elucidate the effects of fluoxetine on other ecologically-relevant behaviors such as burrowing and feeding. Because SSRIs are most commonly associated with municipal wastewater effluents, they generally exist as mixtures rather than individual chemicals in surface waters. It would be prudent to evaluate the potential for surface waters to disrupt neuroendocrine pathways based on total SSRI concentration and activity rather than focus on individual chemicals because the combined total SSRI activity may better estimate effects on critical biological endpoints.

SUMMARY AND CONCLUSIONS

The results from this research demonstrated that fluoxetine has the potential to disrupt reproduction of native mussel species by inducing release of spermatozeugmata from male mussels, inducing release of nonviable glochidia (larvae) from gravid female mussels, as well as altering the mantle flap display behavior of female mussels. The lowest observed effect concentration (LOEC) of fluoxetine in our study (300 μg/L) is greater than those measured to date in the environment (maximum 0.1 μg/L); however, our test durations were relatively short ($48 \leq 96$ h) for all experiments. The LOEC for release of nonviable glochidia by female *E. complanata* in the present study was 300 μg fluoxetine/L, but the next lowest test concentration was 30 μg/L, thus the true threshold for effects (in acute tests) lies somewhere between 30 and 300 μg/L.

To our knowledge this is the first report of reproductive effects with male unionid mussels exposed to an SSRI drug. Male *E. complanata* exposed to 3000 μg fluoxetine/L released spermatozeugmata (aggregates of hundreds to thousands of spermatozoa) but those exposed to the next lowest concentration, 300 μg/L, did not. The effects of fluoxetine on viability of spermatozeugmata or individual spermatozoa after release from the sperm sphere have not been elucidated.

Finally, consistent with other studies of mollusks in which fluoxetine stimulated reproductive processes, fluoxetine exposure resulted in a concentration-dependent stimulation of mantle lure display behaviors by female *Lampsilis fasciola* and *Lampsilis cardium*. It is currently unclear if such behavioral effects would preclude successful attraction of a suitable host fish for glochidia infestation. Additionally, it is not known if fluoxetine (or other SSRIs) would induce display behaviors in female mussels that are not gravid (i.e., out of season), or prematurely induce displays in those that contain immature or nonviable glochidia.

RECOMMENDATIONS

Currently little is known about the effects of chronic, low-level fluoxetine exposure (consistent with surface waters receiving treated wastewater effluent) on freshwater mussel reproduction. Therefore, additional research is needed on the potential long-term effects of fluoxetine on mussel reproduction and behavior, especially in surface waters receiving treated wastewater effluent. Moreover, because freshwater mussels are sediment-dwelling organisms, there remain uncertainties about the potential bioavailability and toxicity of sediment-bound fluoxetine and other SSRIs to mussels. Additional research is also required to elucidate the effects of fluoxetine on other ecologically-relevant behaviors of mussels such as burrowing and feeding. Because the SSRIs are most commonly associated with municipal wastewater effluents, they generally exist as mixtures rather than individual chemicals in surface waters. Thus, it would be prudent to evaluate the potential for surface waters to disrupt neuroendocrine pathways based on total SSRI concentration and activity rather than focus on individual drugs because the combined total SSRI activity in a stream may better estimate effects on critical biological endpoints.

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Restoring Biogeochemical Functions in Degraded Urban Stream Ecosystems

Basic Information

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RESTORING BIOGEOCHEMICAL FUNCTIONS IN DEGRADED URBAN STREAM ECOSYSTEMS

ABSTRACT

A growing body of research demonstrates the important effect stream ecosystems have in altering the form, timing and magnitude of watershed nitrogen (N) losses. Most of this research has been conducted in minimally impacted watersheds. Streams in heavily urbanized watersheds may be functionally disconnected from upland soils, with a high proportion of precipitation routed over pavements and through storm drains directly into channels. Receiving streams, in turn, will become little more than gutters routing stormwaters towards the sea. Urban streams thus represent the worst case scenario, integrating a large number of simultaneous watershed insults. Several very recent studies suggest that these streams have very reduced capacities to transform and retain N. These same studies also demonstrate that N transformation and retention is closely tied to organic matter (OM) dynamics. For the last year we have examined differences between 12 focal stream reaches in the Raleigh-Durham metropolitan area, comparing streams from forested watersheds (n=4) with those in urban watersheds (n=8) in reaches that are degraded (n=4) or recently restored (n=4). We have found that stream restoration efforts do not appear to be restoring habitat or flow heterogeneity. The urbanized streams in our survey tend to have slower flows, more homogeneous substrate, and greater channel incision than their forested counterparts and indeed restored stream reaches are virtually identical to urban streams, with the exception of having reduced channel incision. Our efforts to document differences in ecosystem function across these twelve streams have proven less sensitive. Urbanization tends to shift stream ecosystems towards increasingly productive systems, with higher nutrients, slower flow and higher light levels stimulating algal growth. Restoration projects tend to eliminate riparian trees, thus the major effect of restoration on ecosystem function is warmer, more well lit streams that have higher algal production and higher nutrient uptake than their urban counterparts.

Statement of Critical Water Resources Problem:

High levels of nitrogen are loaded to increasingly degraded streams: Humans have roughly doubled the annual supply of nitrogen (N) to the planet. This has numerous detrimental impacts, including increased fluxes of nitrogen in rivers, leading to excessive nitrogen concentrations, harmful algal blooms, and regional hypoxia in many coastal waters and estuaries (Green, Vorosmarty et al. 2004). The streams that receive these increasingly high nitrogen inputs have a tremendous capacity to transform reactive N (available to plants and microbes) back into inert atmospheric N_2 through biological uptake and denitrification within river sediments (Peterson, Wollheim et al. 2001; Bernhardt, Likens et al. 2003; Bernhardt, Likens et al. 2005). Recent global modeling estimates have suggested that at least half of the nitrogen entering river systems appears to be lost to denitrification on its way to the sea (Galloway, Dentener et al. 2004). The smallest streams are the most effective at nitrogen removal (Alexander, Smith et al. 2000; Seitzinger, Styles et al. 2002), yet many of our smallest streams are poorly protected by current environmental regulations and are heavily impacted by pollution and channelization. Currently, over 130,000 km of U.S. streams are impaired by urbanization (USEPA 2003). This estimate will certainly increase over the next 30 years, as virtually all of the world's population growth is expected to occur in urban areas, with over 60% of the world's population in urban areas by 2030 (UNPD 2003). Urbanization and suburbanization of watersheds results in a series of predictable changes in streams, leading to radically altered channel forms (wide, shallow, straight channels with little depth or velocity variation) and hydrology (high peak flows, reduced base flows, and discontinuity between channel and subsurface sediments (Paul and Meyer 2001). Because urbanization simultaneously increases the loading of sediments and nutrients while simplifying the stream channel, urban rivers are effectively changed from functioning ecosystems to gutters. A number of recent papers demonstrate that urban streams have very reduced capacities for nutrient uptake and retention (Grimm, Crenshaw et al. *In Press;* Groffman and Dorsey *In Press;* Groffman, Law et al. *In Press;* Meyer, Paul et al. *In Press*), yet to date this work been primarily descriptive rather than mechanistic.

Investments in river restoration attempt to reduce N export: Concern over the impacts that land use changes may have on the ability of river systems to provide the ecological and social services upon which human life depends has resulted in the initiation of major investments in urban river restoration (Bernhardt, Palmer et al. 2005). More than one third of all river restoration projects in the U.S. are implemented to "manage and improve water quality", yet these projects are rarely evaluated to determine if this goal is achieved (Bernhardt, Palmer et al. 2005). In urban areas, multi-million dollar projects are aimed at "renaturalizing" these simplified channels back (hopefully) into functioning ecosystems (supporting of diverse fauna and capable of retaining sediments and nutrients) (Bernhardt and Palmer In review).

Related Research and Justification:

Preliminary Data

Recent work has demonstrated that rates of nitrogen uptake in urban streams are low compared to undisturbed systems (Grimm, Crenshaw et al. *In Press;* Meyer, Paul et al. *In Press*), Palmer & Bernhardt, in prep.), and that BOM levels are low (Meyer, Paul et al. *In Press*) and correlated with nitrogen uptake and denitrification (Groffman and Dorsey *In Press;* Meyer, Paul et al. *In Press*).

Efforts to reconnect stream channels with their floodplains and to slow flow via remeandering and placement of channel obstructions should lead to greater opportunities for inorganic nitrogen removal in riparian soils and hyporheic sediments. Preliminary surveys of four channel reconfiguration projects within urban watersheds near Durham suggest that these efforts are attenuating high NO_3 loads.

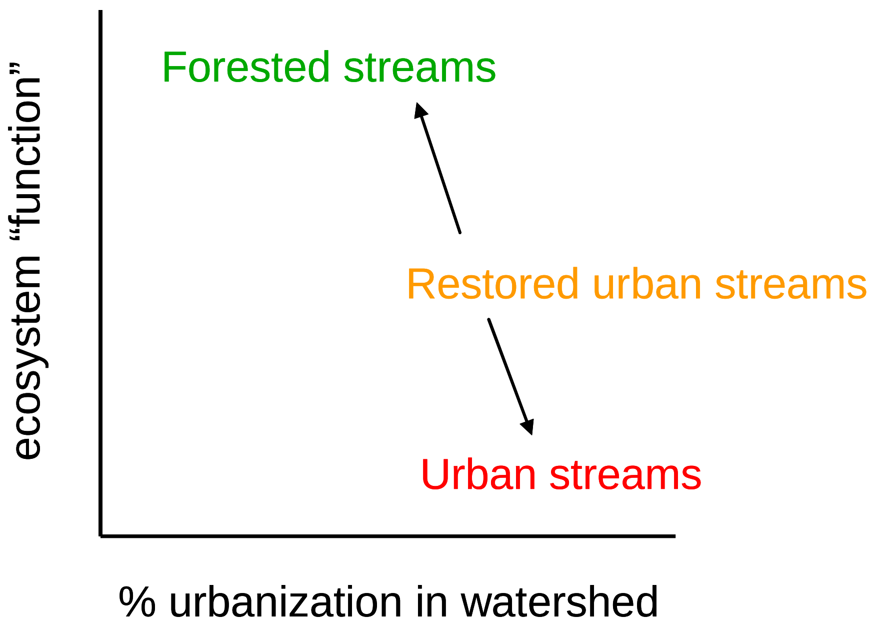
Nitrogen uptake in streams is a function of labile C availability: In the last decade, a growing body of research has documented the important role that stream ecosystems, particularly headwaters, play in influencing the downstream export of nitrogen (Alexander, Smith et al. 2000; Peterson, Wollheim et al. 2001; Bernhardt, Hall et al. 2002; Seitzinger, Styles et al. 2002). Efforts to understand the mechanisms that control nitrogen uptake have been less productive. Indeed, inter- and intra-biome studies of stream N cycling have tended to find that no measured variables are tightly correlated with nitrogen uptake (Webster, Mulholland et al. 2003). Exceptions to this trend are several lines of research that suggest carbon supply and carbon processing (metabolism) are good correlates of nitrogen uptake both within and among streams (Bernhardt and Likens 2002; Hall and Tank 2003; Meyer, Paul et al. *In Press*). Tight linkages between carbon and nutrient processing are expected -- given stoichiometric and thermodynamic constraints on organisms -- yet it can be difficult to conceive of small forested streams as carbon limited. However, carbon can be strongly limiting even in streams with high organic matter standing stocks. Experimental additions of labile carbon to: i) stream sediment samples (Strauss and Lamberti 2000); ii) hyporheic flowpaths (Baker, Dahm et al. 1999) and iii) entire streams (Bernhardt and Likens 2002) have been shown to: i) decrease nitrification; ii) stimulate N assimilation and denitrification; and iii) eliminate inorganic nitrogen export from an entire watershed.

Urban stream microbial activity is likely to be C limited: Geomorphic and hydrologic research in pristine streams demonstrates that structural elements in streams (e.g., debris dams) increase the deposition and storage of fine material(Bilby 1981). In heavily degraded urban streams, most of these structures have been removed, either through active management or due to frequent high, flashy stormflows. These simplified channels thus have a reduced capacity to trap organic matter (OM) and their beds are frequently scoured – reducing levels of deposited organic material as well as attached periphyton. Organic matter serves not just as an energy source but as a substrate for microbes; therefore, carbon limitation is likely to be particularly severe in urban stream ecosystems. This is unfortunate, since these same streams typically receive high nitrogen inputs (Paul and Meyer 2001; Groffman, Law et al. *In Press*). Even when urban impacted streams have intact riparian forests (and thus levels of litter inputs comparable to undeveloped sites), they will have a dramatically reduced capacity to retain these materials within the stream channel due to simplified channel structure and flashy storm hydrographs. Because restored streams frequently are engineered for increased channel complexity and sinuosity, they have the potential to increase OM retention and metabolism

STUDY OBJECTIVES

We predicted that streams in urban watersheds would have simplified habitat structure and be impaired in ecosystem function relative to their minimally impacted counterparts in predominantly forested watersheds (Figure 1). We also predicted that restoration efforts would lead to stream ecosystems that fell out intermediate in both structural and functional attributes relative to forested and urban watershed streams.

Figure 1: Hypothetical predictions for the effects of urbanization and restoration on stream ecosystem function



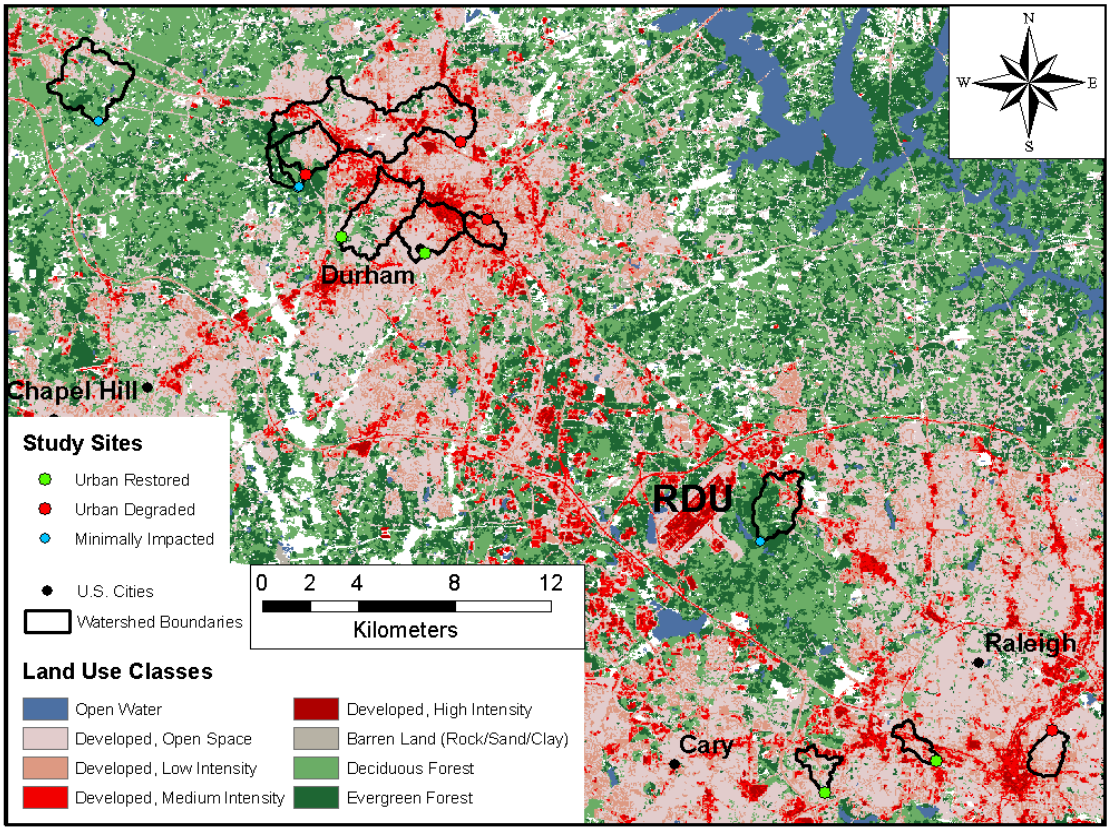
We examined these predictions through detailed comparison of 12 stream reaches distributed between 3 categories: forested watersheds (4 streams draining watersheds that were minimally impacted by urban development); urban degraded streams (4 streams draining heavily urbanized watersheds without any channel restoration); and urban restored (4 streams draining heavily urbanized watersheds that have undergone some form of natural channel design river restoration within the last eight years). For this set of 12 streams we made the following set of predictions in our original proposal (Table 1). In each case, we predicted that these factors would differ between the forested and the urban stream reaches, and hypothesized that successful restoration would lead to measurements that were intermediate to the urban and forested endpoints.

Table 1. Response Variables	Developed relative to undeveloped	
	Mean	Variance
Hydrologic		
Storm pulse amplitude	>	na
Transient storage	<	na
Hydraulic connectivity	<	<
Geomorphic		
Channel Incision	>	<
Water depth	<	<
Channel width	>	<
Biogeochemical		
Benthic Organic Matter (BOM)	<	<
Community Respiration (CR)	<	<
Denitrification potential (DEA)	<	<
Microbial biomass	<	<
DIN uptake velocities	<	<
Nitrification	>	<

STUDY SITES

We set up a comparative study of streams from 12 watersheds within the Raleigh-Durham metropolitan area (see Figure 2). Four streams were in predominantly forested watersheds (<10% impervious cover) with our study reaches at least a kilometer downstream of any impervious cover (impacts in headwaters) (Table 1). Eight “urban” streams drained watersheds ranging from 11-40% impervious cover (Table 1). Four of our study reaches within these urban streams had been restored within the last decade and were recommended as the “best case scenarios” for restoration by staff of the NC EEP and the NC Stream Restoration Institute. In each stream we located an intensive study reach that was representative of local conditions and which allowed at least one hour of water travel time during summer baseflow. In the restored streams we chose reaches at the downstream end of the restored segments, operating on the assumption that these segments would benefit from both local and upstream effects of the restoration project. Our goal in this study was not to examine the average restoration project, but instead to examine the potential for restoration to achieve habitat improvement or ecosystem functional benefits, thus we chose the projects and the reaches that we expected would maximize restoration benefits.

Figure 2: A map showing the distribution of study sites by land use category. Note that even our minimally impacted "forested" watershds have some level of



SAMPLING METHODOLOGY

This research program will focus on measuring A) stream metabolism and inorganic nitrogen uptake in a series of degraded and restored urban streams as well as several reference streams (n=4 of each) and relating these vital ecosystem functions to two key structural attributes of stream channels; B) hydraulic connectivity between the stream channel and its riparian zone and between surface water and hyporheic groundwater; and C) organic matter retention and storage. We request funding for the initial year of research, but anticipate pursuing renewal funding from WRRI and additional funds from other sources (e.g., NSF, EPA, NC EEP) to continue this research for at least three years.

Functional Measures: Metabolism and Nitrogen Uptake

Metabolism: Ecosystem metabolism is an expression of all heterotrophic and autotrophic activity in the stream and thus would be expected to be influenced by any change in shading, allochthonous input, thermal regime, or nutrient concentrations due to urbanization or stream restoration. Restoration efforts should slow streamflow and increase transient storage of surface water and exchange with hyporheic and shallow groundwater reservoirs. The resulting increase in water-sediment contact time and depositional habitats should lead to higher net ecosystem metabolism rates. Although metabolism rates may not be linearly affected by urbanization, ecosystem metabolism has been shown to control ammonium uptake in both relatively pristine (Hall and Tank 2003) and urban streams (Meyer, Paul et al. *In Press*).

Methods: Gross primary production (GPP), community respiration (CR), and net ecosystem metabolism (NEM=GPP-CR) will be estimated using the two-station method described by (Marzolf, Mulholland et al. 1994). This method uses oxygen probes at the top and bottom of a reach to measure oxygen change over the reach, and a propane and conservative tracer release to estimate transit time and oxygen exchange rate. We will also measure redox potential and respiration, using respiration chambers and redox probes, to determine the status of heterotrophic metabolism in riparian soils and hyporheic sediments.

Expected Results: Little structure and frequent disturbance due to flashy floods may limit the algal population in the urban streams, limiting GPP, and these effects may not be mitigated in the restored streams. CR is associated with stable, organic substrate, such as leaf packs, so we expect CR to be correlated with in-stream benthic organic matter. Naturally occurring stream complexity in the reference streams, and increased structure in the restored streams, will lead to larger transient storage zone volume, which could increase NEM.

Nitrogen Uptake

Whole-stream uptake: We will use standard methods(Newbold 1981; Bernhardt, Hall et al. 2002) to measure the rate at which inorganic nitrogen is removed from the water column. Briefly, we will perform back to back co-injections of $NaNO_3$ then NH_4Cl with a hydrologic tracer (NaBr then NaCl). We will examine the downstream change in the concentration of the nutrient relative to the inert tracer. We will use the slope of the decline for each release to estimate, NH_4, NO_3 and total nitrogen uptake rates and whole-stream nitrification.

Riparian and Hyporheic Denitrification Rates: Denitrification is the only process by which nitrogen can be permanently removed from the stream channel and is thus the critical biogeochemical function that we would like to promote within restored stream reaches. We will measure denitrification potential by incubating stream and riparian sediment samples from each reach (**Groffman**, **Holland** et al. 1999). We will compare rates between streams to determine whether urbanization and/or restoration affects denitrification rates. We will also examine the relationship between BOM and denitrification potential for individual cores. In one representative stream within each category, we will supplement these estimates by measuring *in situ* denitrification rates in riparian and hyporheic sediments using ^{15}N single-well push-pull tests (Addy, Kellogg et al. 2002). Briefly, groundwater is extracted from a riparian or hyporheic well, supplemented with $^{15}NO_3$ along with hydrologic (NaBr) and gas (propane) tracers, and returned to the well. Samples are removed from the well 1, 3 and 8 hours following the injection and analyzed for NO_3, N_2O, Br, propane and $\delta^{15}N$ of NO_3 and N_2O. This technique provides a direct measure of biological uptake of labeled NO_3-N, as well as production rates of N_2O through denitrification.

<u>Structural Measures: Hydraulic connectivity and organic matter storage</u>

Stream Hydrographs: We have continuously monitored stream height in all streams by installing a pair of datalogging Hobo© pressure transducers at the upstream end of each reach *[these were purchased with funds from the NC EEP Monitoring and Research program]*. We are still working to develop rigorous flow rating curves for each reach by calculating changes in instantaneous flow throughout at least one storm event in each stream (more rigorous rating curves will be developed through time, but are beyond the scope of this one year study). The stream height data will be used to calculate daily, seasonal, and annual flow statistics (e.g., flood frequency and magnitude, and "flashiness").

Hydraulic connectivity: We consider hydraulic connectivity to be maximum in streams with: 1) less incised channels; 2) more variable water table depths (in riparian zone) and vertical hydraulic gradients (in channel); and 3) movement of solutes between riparian, hyporheic and surface water.

1) *Channel Incision*: We worked within the existing monitoring framework of the NC Ecosystem Enhancement Program (NC EEP) to assess channel incision by measuring bankfull channel shape and dimension at 5 transects in each study stream(Pizzuto, Hession et al. 2000). We also determined channel slope, grain-size distributions, channel sinuosity and created detailed habitat maps for each reach. These physical measurements are made annually by NC EEP for each of the restored streams in our survey. Thus we utilized many of the same approaches for the other 8 streams.

2) Movement of Solutes Between Channel and Subsurface: At each study site we conducted solute tracer releases once in summer 2006 and again in winter 2007, and continuously record solute breakthrough curves in the water column (to estimate water residence time and physical water storage) (Jones and Mulholland 2000).

Organic Matter: Organic matter (OM) in streams serves many functions, but it is especially important as a carbon source for the ecosystem. As a food source for macroinvertebrates, it serves as the base of the food web. As a food and substrate source for bacteria, algae, and fungi, it supports the ecosystem function of water quality improvement which these organisms provide. In particular, fine benthic organic matter (FBOM) in streams has been shown to be a highly correlated with nitrogen removal. In urban systems, OM levels are very low due to both reduced inputs from the riparian zone and reduced retention in the stream(Paul and Meyer 2001). Because organic matter is a cornerstone of several ecosystem functions which stream restoration targets, it could serve as a proxy for those functions in post-construction monitoring.

The first step in understanding OM dynamics in urban streams is to quantify the existing levels. In summer 2006, we sampled 10 transects for each study reach. All coarse benthic organic matter (CBOM) was first removed from 1-m long transect across the full width of the streambed at each transect. After surface CBOM was removed, a core sampler was inserted into the stream bed to measure FBOM, by mixing sediments to 10cm depth within the sampler, recording the volume within the core and removing a subsample. Each sample was weighed in the field and subsamples were returned to the lab. We characterized each sample for % wood and % leaves. All samples or subsamples were subsequently dried and ashed. This allows us to estimate both total dry mass and total ash-free dry mass for the stream bed CBOM and FBOM.

MAJOR RESULTS

ECOSYSTEM STRUCTURE

Hypothesis 1: Streams in urban watersheds will have higher peak flows during similar storm events compared to their forested counterparts.

We are still in the process of working through all of the hydrologic datasets for our 12 streams, but we clearly see reduced peakflows in our reference streams as compared to their urban counterparts. Shown here (Figure 3) is a comparison of two storm hydrographs for one urban and one forested stream within our survey. These streams are immediately adjacent and receive identical levels of precipitation, yet the urban stream hydrograph rises more rapidly and to a greater amplitude than its forested counterpart.

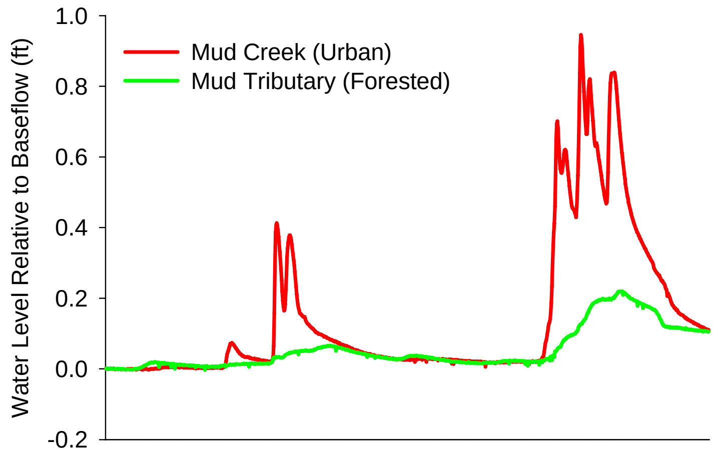


Figure 3: A comparison of two April 2007 storm hydrographs between Mud Creek (an urban watershed) and its full forested tributary.

Hypotheses 2&3: Streams in urban watersheds will have significantly lower levels of hyporheic storage of water and hyporheic exchange (as measured through transient storage modeling of conservative tracer releases) as compared to their forested counterparts.

We were surprised to find no differences in the transient storage metrics between our minimally impacted and urban streams from our 12 stream survey (Figure 4). We hypothesize that urbanization leads to dramatic reductions in hyporheic exchange and storage (through sedimentation and packing of interstitial spaces in the streambed) but simultaneously leads to large increases in within channel storage through the creation of streambeds that are low gradient, deeper and consisting primarily of slow moving pools and runs. This hypothesis is borne out in our geomorphic data (below), and the inability of current hydrologic models to resolve the differences between in channel and hyporheic storage is a commonly acknowledged flaw.

Similarly, because our model failed to differentiate between exchange within the channel (between fast and slow moving compartiments) and between the channel chan the subsurface (hyporheic exchange) we were unable to find any significant differences between stream types in hyporheic exchange (Table 3).

Figure 4: Examples of conservative tracer breakthrough curves and model fits for calculating transient storage metrics for one block of stream reaches are shown above a summary graph showing the lack of differences in the % of total stream volume in transient storage (A_s/A) across stream types.

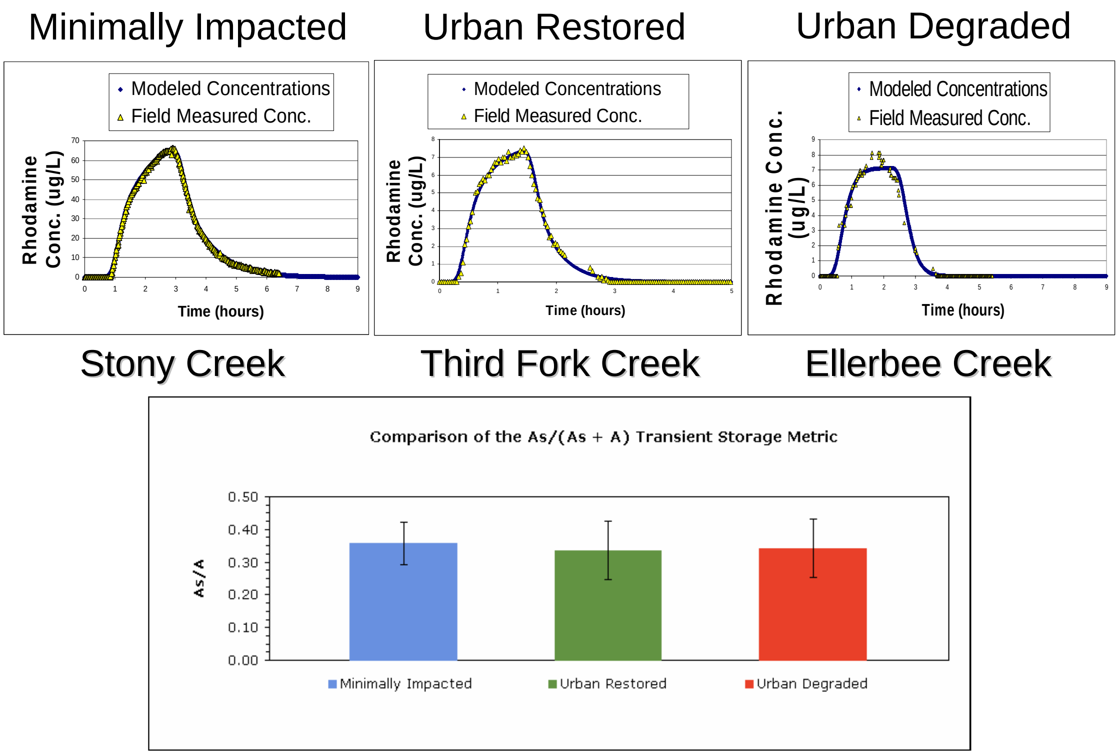
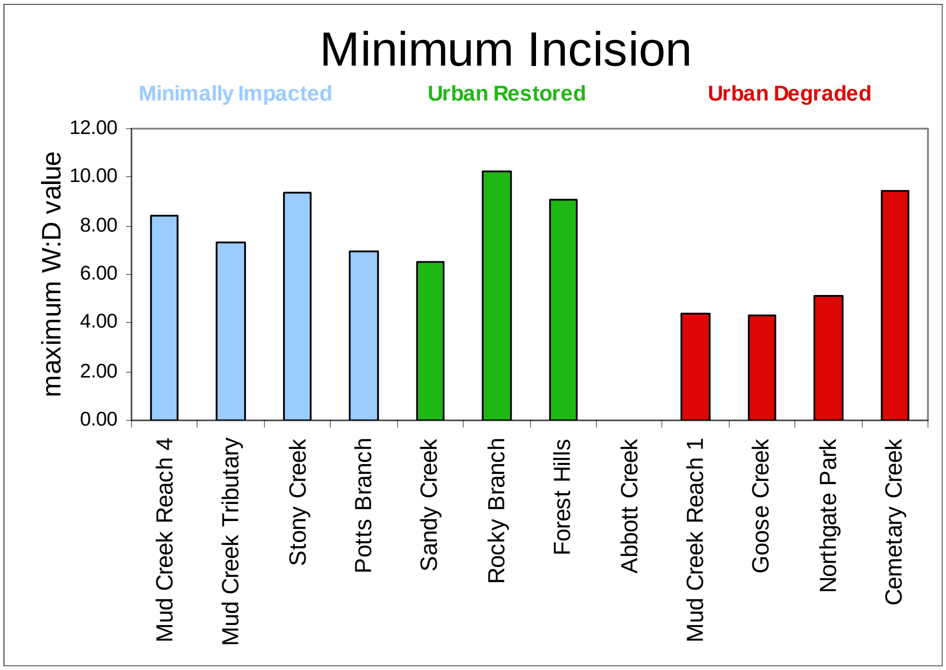


Table 3. Transient Storage Metrics

Block	Status	Site Name	H	A_s/A	Fraction of Median Travel Time Due to Transient Storage (F_{med}^{200})
A	Forested	Stony Creek	3.00E-04	0.714	41.65%
	Restored	Forest Hills	8.71E-04	0.894	47.11%
	Urban	Northgate Park	1.50E-03	0.201	14.37%
B	Forested	Potts Branch	9.30E-04	0.422	28.53%
	Restored	Abbott Creek	1.10E-04	0.451	30.98%
	Urban	Cemetary Creek	1.00E-04	0.780	43.70%
C	Forested	Mud Creek Tributary	5.90E-03	0.305	21.52%
	Restored	Rocky Branch	5.80E-04	0.337	43.59%
	Urban	Goose Creek	1.00E-04	0.752	42.91%
D	Forested	Mud Creek Reach 4	4.20E-04	0.937	48.37%
	Restored	Sandy Creek	9.50E-04	0.285	19.52%
	Urban	Mud Creek Reach 1	9.80E-04	0.501	33.21%

Hypothesis 4: Streams draining urbanized watersheds will have higher degrees of channel incision than their forested counterparts, indicating less exchange of water and materials between the stream and its surrounding riparian zone.

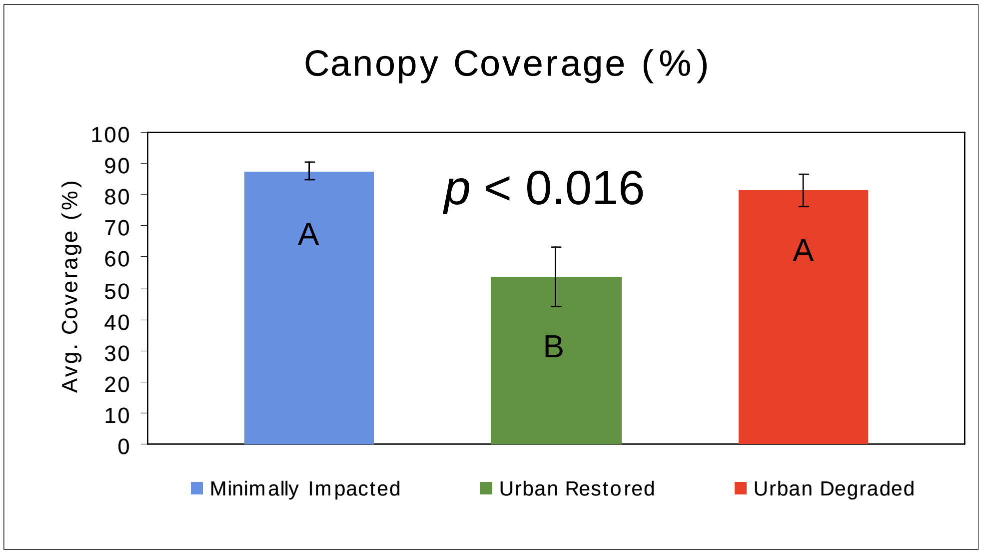
Figure 5:Measures of channel width:depth ratios for 11 of our 12 streams. For this metric, higher width:depth ratios are indicative of less incised streams



We found that with the exception of Cemetary Creek (a stream which is bedrock constrained throughout our study reach and for which channel incision is thus not possible) urban streams were more incised than either their forested or restored counterparts (Figure 5). Because creating less incised channel cross sections are one of the main goals of most natural channel design stream restoration projects it is not surprising to see that restoration does lead to decreased incision. However, because these projects have gone through several seasons or years since implementation, it is encouraging to find that the channels have held their new channel shape.

Despite the increased opportunity for hydrologic connectivity between restored urban stream channels and their riparian zones, we noted a discouraging trend towards dramatically reduced riparian canopy cover in all of our restored streams (Figure 6). In many cases mature trees were removed from the riparian zone in order to facilitate bank regarding and channel reconfiguration, leading to stream channels that have less canopy shading and reduced inputs of riparian leaf and wood material.

Figure 6: Canopy cover over stream reach, measured with densitometer reading throughout each study segment.



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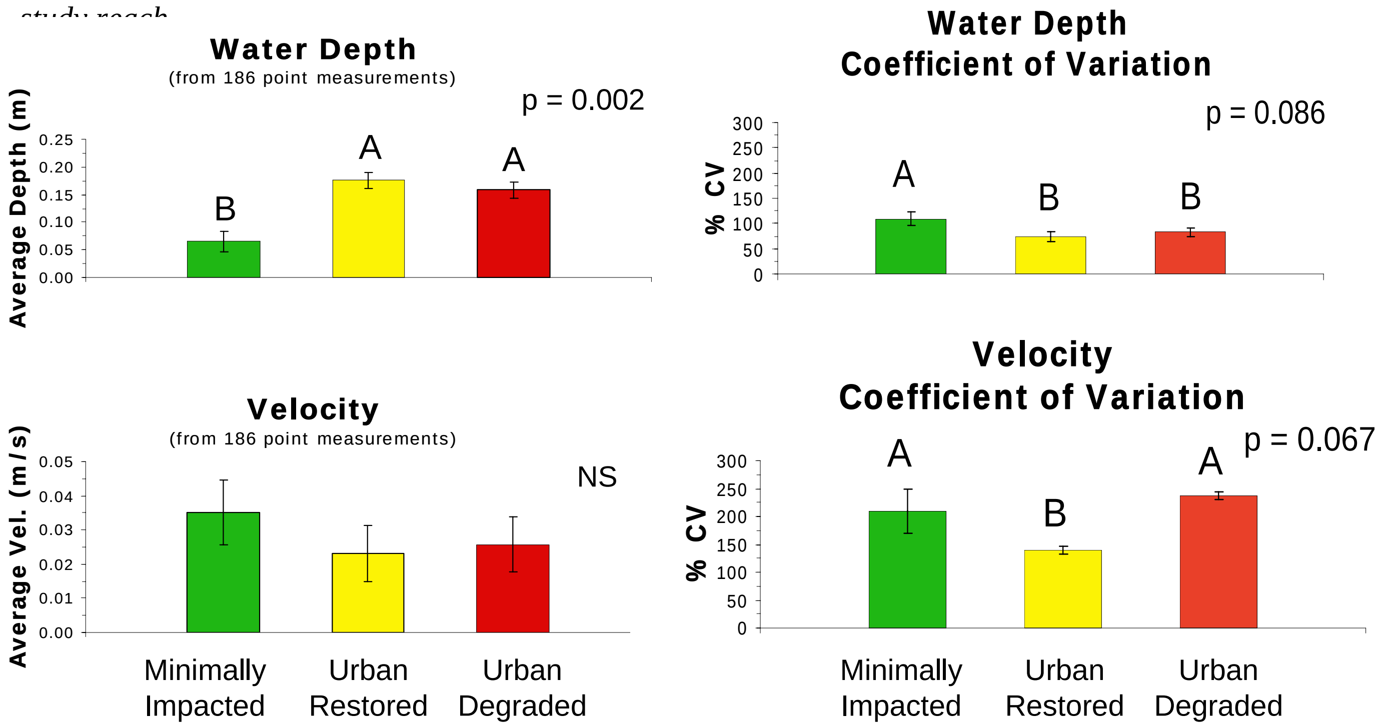
Hypotheses 5&6: We predicted that streams draining urban watersheds would have wider channels with shallower and less variable flow habitats.

We found no significant effects of urbanization on channel width (Table 4), although the wetted width of urban streams tended to be wider than their forested counterparts with similar drainage area. Urbanization tended to lead to streams with shallower channel slopes, deeper water columns with less variable depths and flows (Table 4 and Figure 7).

Table 4. Additional Site Measurements

Site Characteristic		Forested	Restored	Urban
Estimated Discharge (L/s)		5.1 ± 3.0	4.8 ± 1.5	8.7 ± 2.5
Percent Dilution(%)		-82.3 ± 14.8	-103.6 ± 29.3	-69.7 ± 11.9
Flow Velocity (m/s)		0.02 ± 0.002	0.02 ± 0.009	0.01 ± 0.005
Active Channel Width (m)	Avg	4.1 ± 0.7	3.2 ± 0.4	5.4 ± 1.2
	% CV	22.7 ± 5.0	19.7 ± 4.8	18.0 ± 2.0
Wetted Width (m)	Avg	2.7 ± 0.5	2.5 ± 0.3	4.1 ± 1.5
	% CV	34.0 ± 2.7	28.4 ± 6.1	27.7 ± 5.8
Longitudinal Slope (%)	Avg	1.4 ± 0.7	0.3 ± 0.1	0.8 ± 0.6
Degree of Incision D:W)	Avg	0.17 ± 0.02	0.17 ± 0.03	0.22 ± 0.01
	% CV	35.5 ± 11.5	13.5 ± 2.6	17.0 ± 5.3

Figure 7: Average and coefficient of variation of surveyed depths and velocities across stream types. In each stream we measured depth and vel
study reach



These results led to some of the most interesting finding of our study that we thing are relevant to current restoration practices. Although natural channel design approaches are leading to reduced channel incision, they are not recreating the fine scale flow habitat variation that is characteristic of less disturbed watershed streams. Indeed habitat maps were made for each study reach and we documented dramatic differences in the extent and variation in flow habitats between our forested and urban stream categories, with restoration efforts having no measurable effect on habitat heterogeneity.

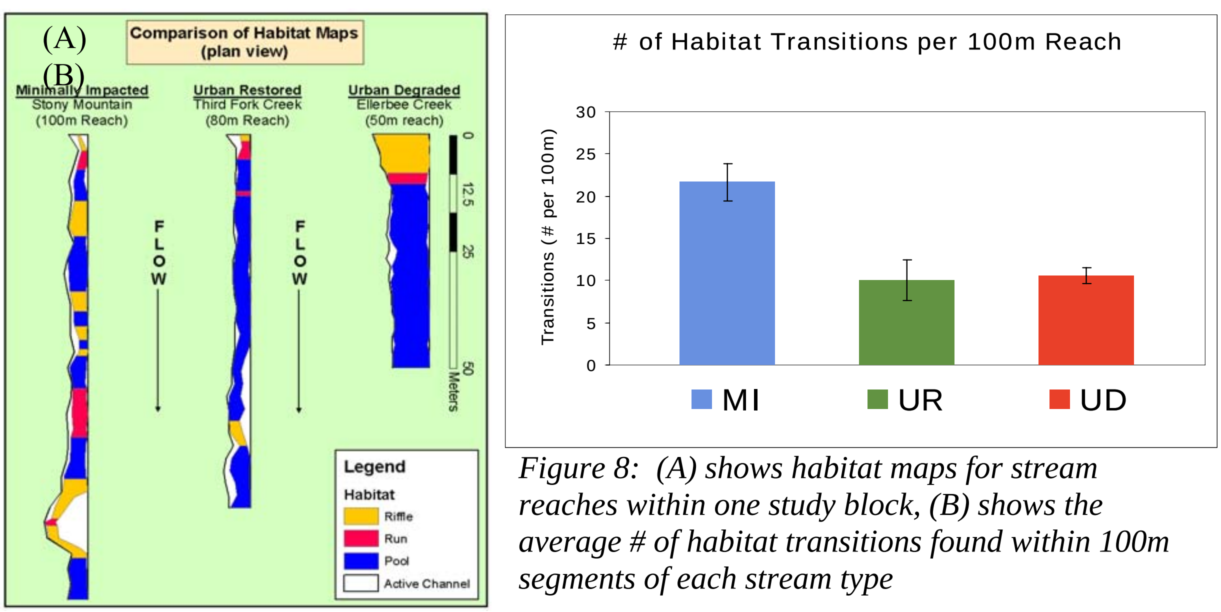


Figure 8: (A) shows habitat maps for stream reaches within one study block, (B) shows the average # of habitat transitions found within 100m segments of each stream type

ECOSYSTEM FUNCTION

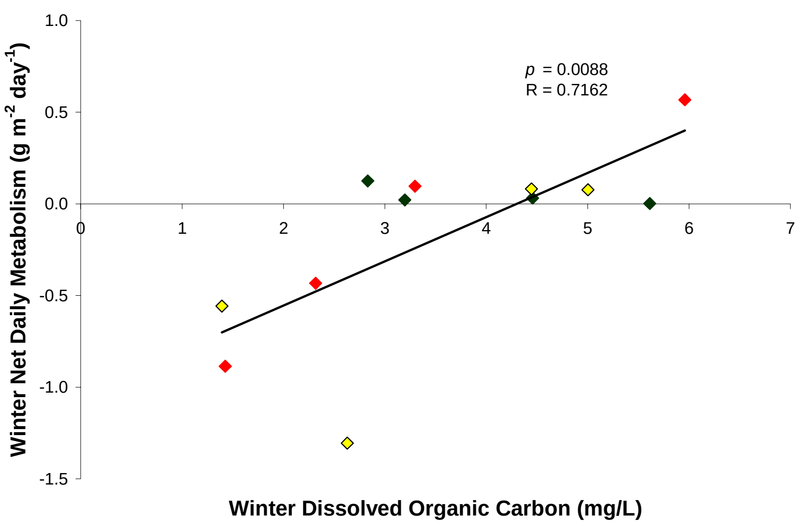
Hypothesis 7: Benthic organic matter stocks will be lower and less variable in urban streams as a result of less retentive habitats to store organic materials as well as flashier hydrographs that significantly increase organic matter losses.

We are still completing the analyses of benthic sediment samples in order to adequately understand the differences in organic matter storage between streams. We did not find significant differences in the standing stocks of surface organic matter (leaves and twigs collected off the stream bed) between our land use categories. However we did find significantly higher reach scale variation in where these materials were distributed within forested stream reaches than in their urban counterparts where surface OM tended to be evenly distributed. We are still waiting to determine the amount and quality of carbon stored within the streambed and expect to have those data by August 2007.

Hypothesis 8: We expected Community Respiration to be reduced in urban streams as a result of dramatic reductions in organic matter storage.

We were surprised that we were unable to detect significant differences in metabolic rates between our land use categories. Although restoration reaches tended to have higher rates of gross primary production these differences were not significant. We did find that winter rates of net daily metabolism (NDM) (NDM = NPP + CR) were strongly driven by water column DOC, a pattern driven almost exclusively by our urban stream datapoints (Figure 9). This correlation suggested to us that much of the metabolism of these urban streams may be driven by labile dissolved carbon in the water column, either produced by algae within these streams, or delivered from leaky sewer pipes and organic matter rich storm sewer outflows (as has been found for urban streams in Baltimore, MD (S. Kaushal, Baltimore LTER, personal communication)

Figure 9: Relationship between DOC and NDM Winter 2007



Hypotheses 8&9 We expected denitrification potentials and microbial biomass to be reduced in urban streams.

Sediment samples for these calculations have recently been collected and these data will be available by August 2007. We have no results to report at this time.

Hypothesis 10 We expected to see higher efficiencies for nitrogen removal from the water column in our forested streams relative to their urban counterparts.

Through repeated sampling we have established that the concentrations of both total and dissolved nitrogen is always higher in our urban streams than in their forested counterparts, with nitrogen concentrations in our restored stream reaches intermediate to these endpoints (Figure 10 A&B).

Figure 10: Monthly streamwater nitrate concentrations across our 12 stream survey. On all but one date (Nov 2006), urban streams had significantly higher nitrate concentrations than forested streams.

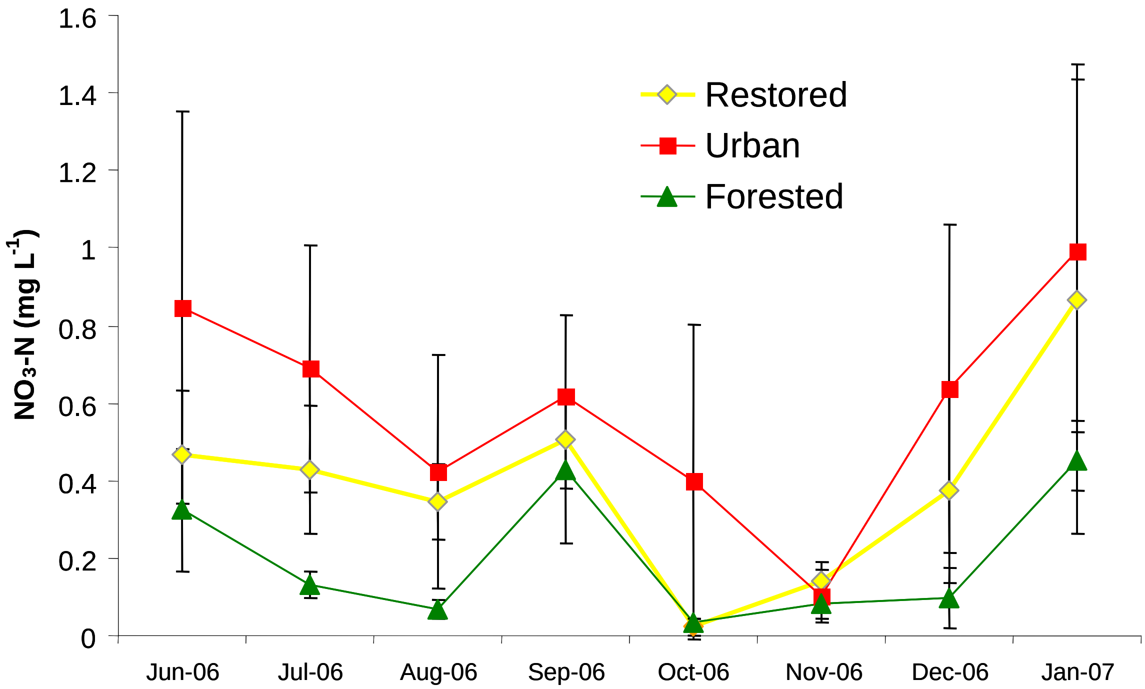
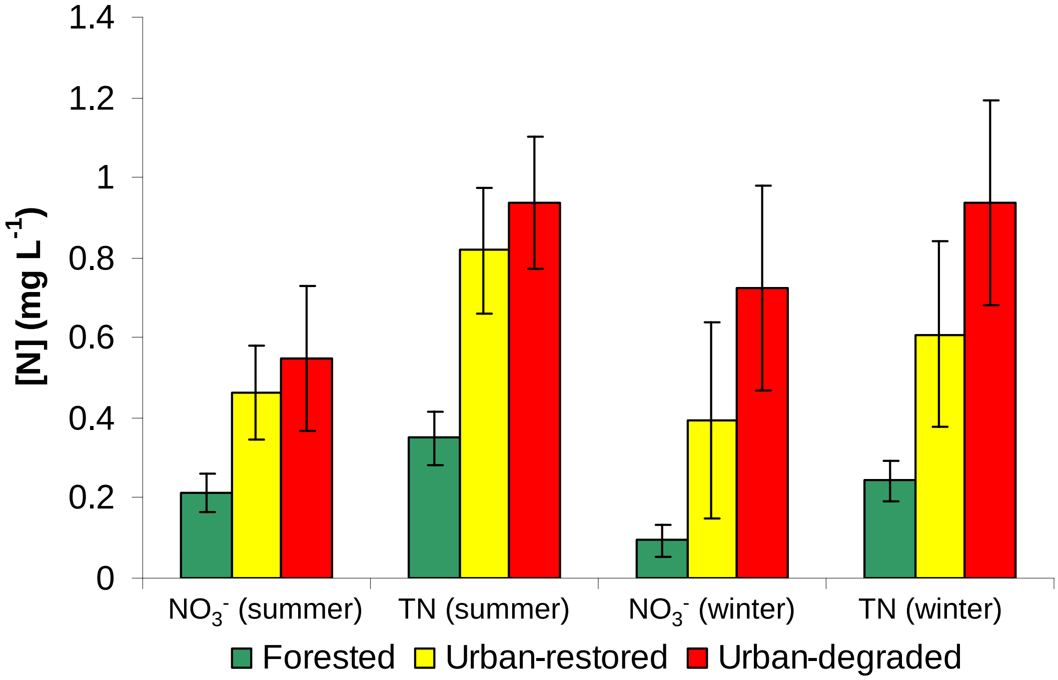
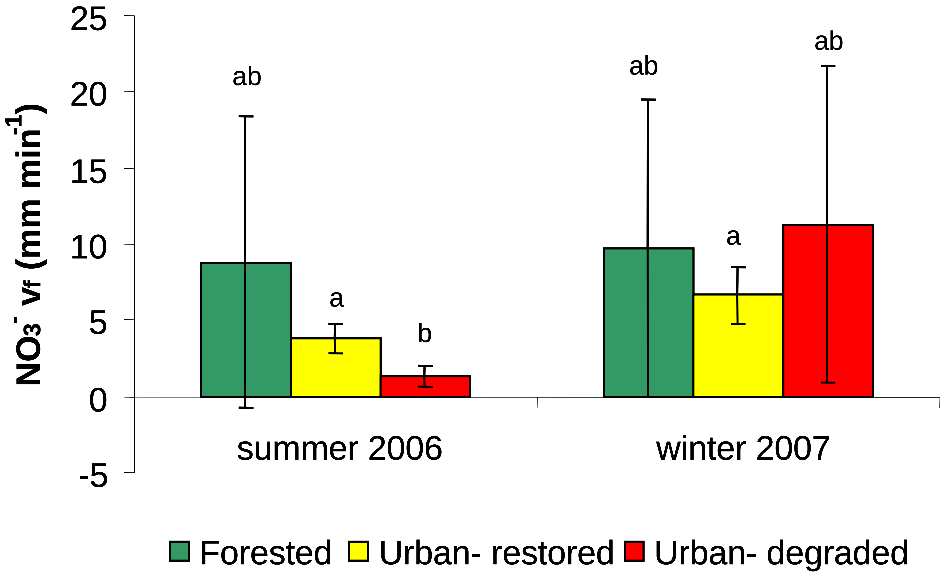


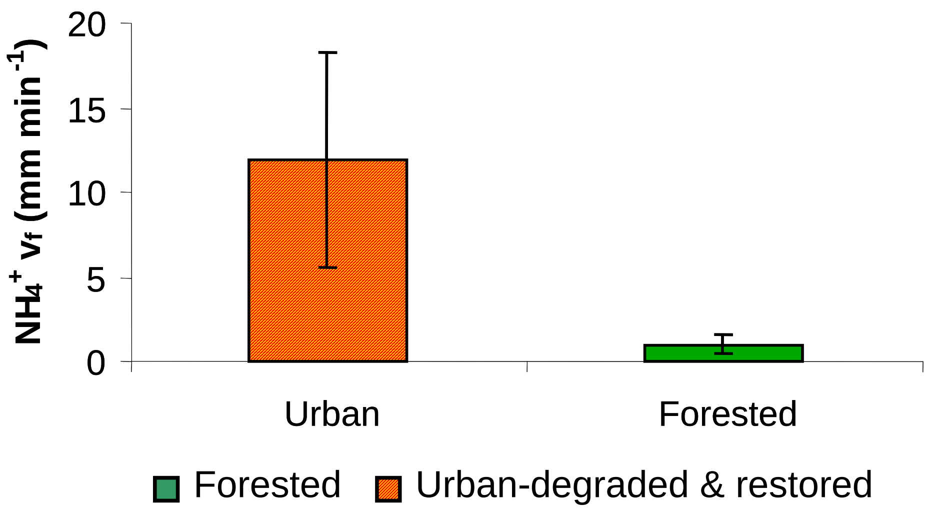
Figure 11: Streamwater NO_3^- and Total Dissolved Nitrogen concentrations as measured immediately prior to nutrient enrichment experiments in summer 2006 and winter 2007



Contrary to our expectations there were no significant differences in nitrate uptake velocities between our forested and urban streams in either seasonal survey (although our restored reaches had significantly higher NO_3^- uptake efficiencies than their urban degraded counterparts during the summer months) (Figure 11A). In contrast to our expectations we found significantly higher uptake efficiencies for NH_4 in both sets of urban streams (degraded and restored) as compared to our forested watershed streams (Figure 11B).

Figure 12: (A) Summer vs. winter nitrate uptake velocities (the speed with which nitrate moves from the water column into the sediments) compared between land use categories. (B) Winter NH_4^+ uptake velocities are compared between the merged urban streams (no differences between restored and degraded sites) and their forested stream counterparts.





Hypothesis 11: We expect rates of nitrification to be higher in urban streams where supplies of both NH_4 and NO_3 are higher.

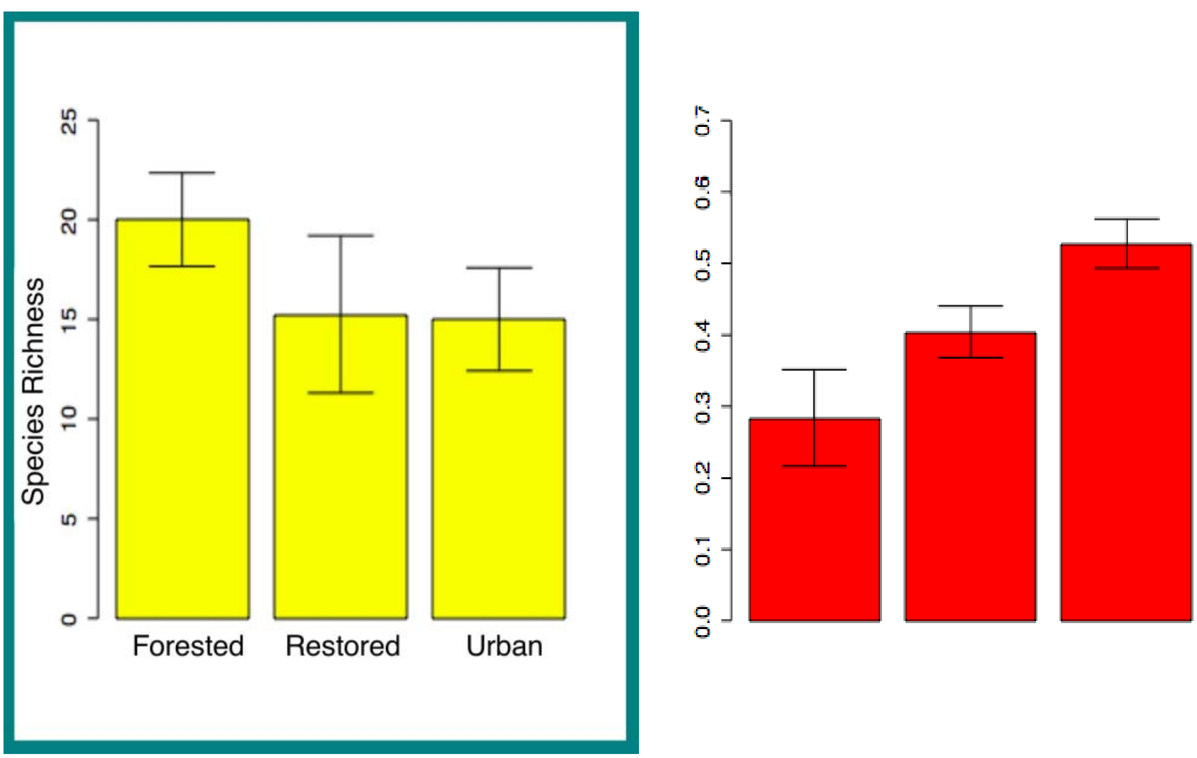
We are still working on the modeling aspect of answering this question from our winter releases, but preliminary data suggests that this hypothesis is correct. In many of our urban streams we see ambient increases in NO_3^- concentrations through our study reaches, providing some evidence that nitrification is generating NO_3^- within the reach. We expect this data analysis to be completed by August 2007.

BIOTIC RESPONSES:

In work complementary to the efforts described here, UNC PhD student Christy Violin has been examining macroinvertebrate communities in each of our 12 study stream reaches. In summer 2006 she used the NCDWQ Qual-4 protocol to sample benthic macroinvertebrates in each stream. We predicted (as for the full study) that urban degraded stream reaches would have much lower macroinvertebrate diversity, and fewer sensitive taxa than their forested counterparts. We hoped that restored streams would fall out intermediate between the forested and urban degraded stream reaches.

Violin used several metrics for comparison. The first is a simple comparison of species richness (Figure 13). Although species numbers were not significantly different between sites, a variety of very tolerant chironomidae (midge larvae) made up the bulk of the species numbers in both the urban degraded and restored streams.

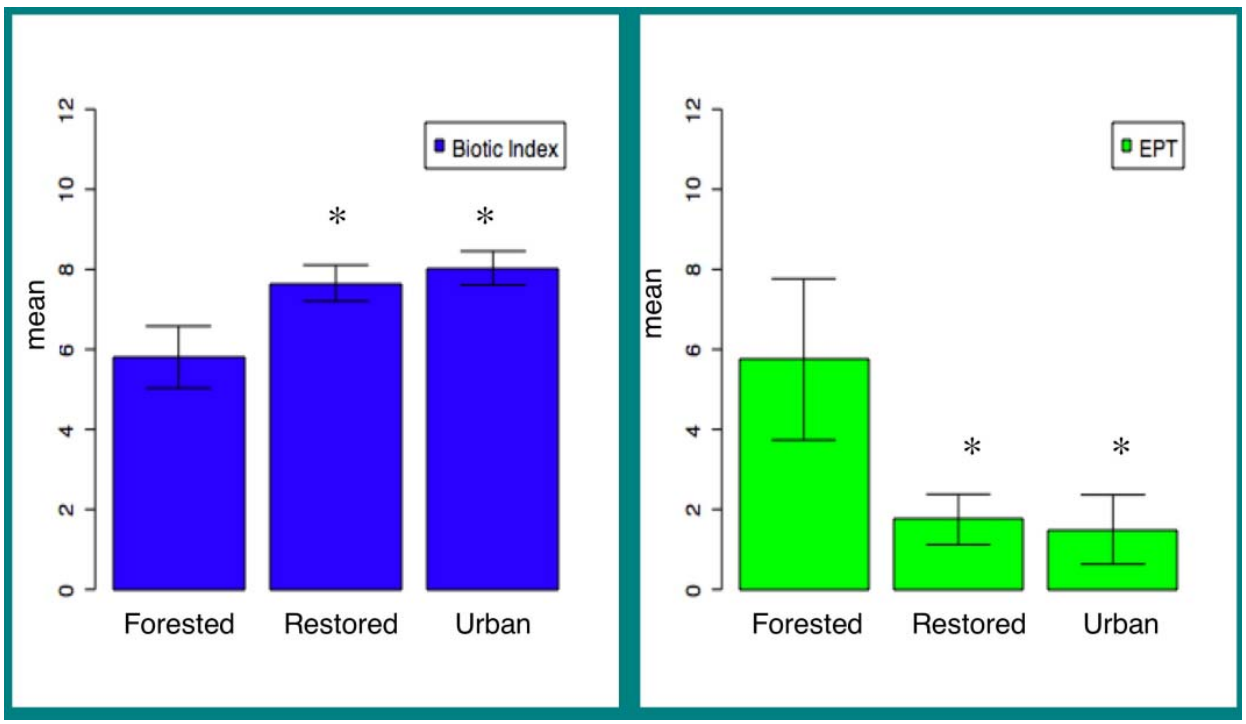
Figure 13: (A) Species Richness (B) The proportion of total species #'s that are due to representatives of tolerant taxa of Chironomidae larvae



Comparisons are much clearer when examining two commonly used macroinvertebrate indices that are established as good correlates of water quality. The first is the EPT index which represents the total number of taxa within the Insect orders of Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies), represented by a wide diversity of organisms with varying life history strategies but typically sensitive to habitat and water quality degradation. Higher EPT scores are indicative of "healthier" communities. The IBI or Index of Biotic Integrity index, is calculated by summing the density of each taxa found in the community multipled by its tolerance value. High IBI scores indicate a community that is dominated by extremely tolerant taxa and are typical of polluted streams and rivers.

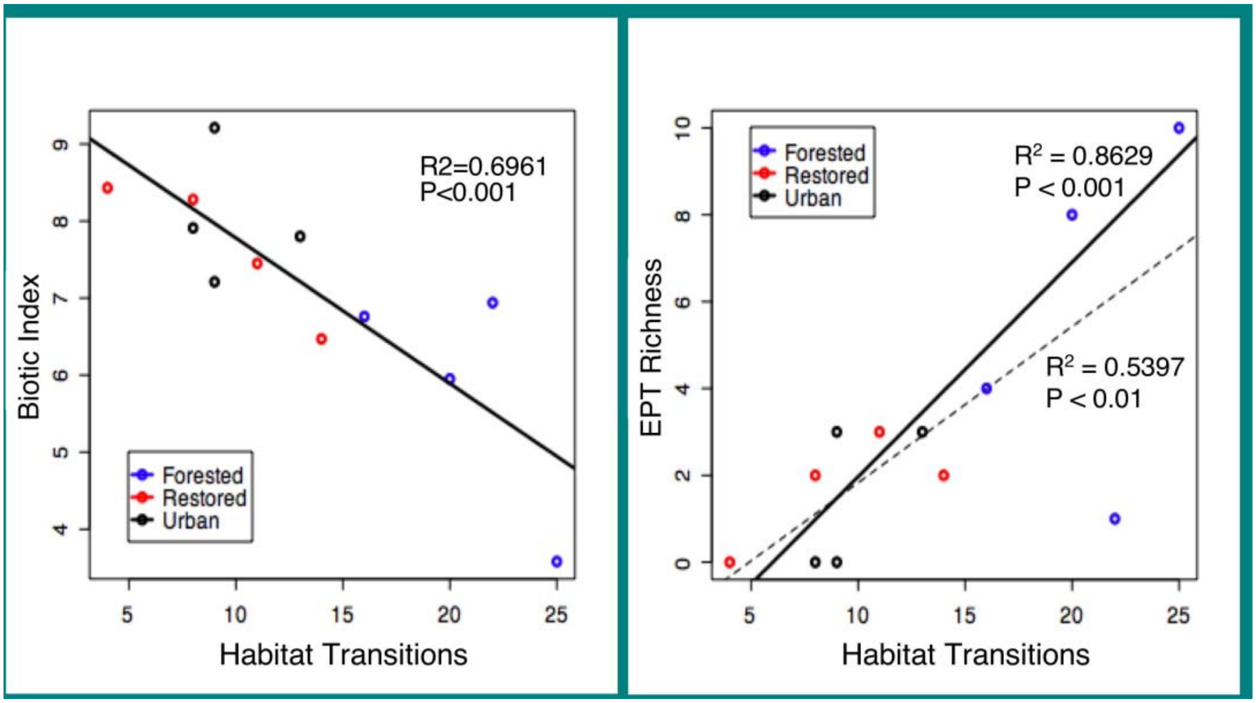
For both IBI and EPT indices, our forested stream reaches scored significantly better than either the urban degraded or urban restored streams, indicating they contain significantly more sensitive species of macroinvertebrates (Figure 14). There were no differences between the urban restored and urban degraded streams (Figure 14).

Figure 14: Macroinvertebrate communities differ among stream types in both (A) IBI scores and (B) EPT scores



Violin also found that the single best predictor of both IBI and EPT scores for each stream reach was the diversity of habitat transitions (as shown in Figure 8 above) (Figure 15). Note that there is a significant correlation for the relationship between IBI and EPT vs. habitat transitions for the 4 restored streams alone, suggesting that some projects are more successful than others at reestablishing this critical variation and supporting a macroinvertebrate community that is closer to "reference" conditions.

Figure 15: Habitat Complexity and Macroinvertebrate Community Composition



Biotic Index EPT

SUMMARY AND CONCLUSIONS

Approach: With the help (and additional financial support of ~$21K) of the NC Ecosystem Enhancement Program we identified 6 urban streams included in their existing program, four previously restored projects and 2 soon to be restored degraded urban streams. These 6 streams, along with 4 reference streams in Umstead Park and the Duke Forest and 2 additional urban streams (one in Raleigh and one in Durham) make up our set of 12 intensive field sites. Within the project period we have: (1) monitored water chemistry once monthly at all 12 streams; (2) developed GIS watershed analyses of land use for the watershed draining to each study reach; (3) performed nutrient injection experiments, measured whole ecosystem metabolism, and modeled transient storage in each reach using low level experimental enrichments of nutrients and hydrologic tracers (each of these measurements were made for each stream in June 2006 and February 2007); (4) conducted a detailed survey of stream and riparian morphology; (5) installed continuously recording water level sensors to develop hydrographs for each site and (6) intensively sampled benthic organic matter at all 12 sites.

Objectives: Our comparison of these 12 stream reaches was motivated by a desire to understand: (1) how urbanization changes both the structure (habitat heterogeneity, hydrologic connectivity, riparian characteristics) and function (metabolism, nutrient uptake) of stream ecosystems; and (2) the extent to which interventionist restoration approaches that use natural channel design to re-engineer degraded channels can move degraded urban ecosystems back towards “reference” conditions.

Findings: We are still in the midst of working up the entire dataset, and expect to submit at least two manuscripts arising from this work in fall 2007. One manuscript will focus on the structural and hydrologic changes in stream channels associated with urbanization and will report our findings that stream restoration efforts do not appear to be "restoring" habitat or flow heterogeneity. The urbanized streams in our survey tend to have slower flows, more homogeneous substrate, and greater channel incision. Restored streams are virtually identical to urban streams, with the exception of channel incision, likely reflecting the focus by restoration practitioners on channel geometry rather than habitat quality. A second manuscript will report our findings on nitrogen processing and metabolism across this urbanization gradient. Urbanization tends to shift streams towards increasingly productive systems, with higher nutrients, slower flow and higher light levels stimulating algal growth. Restoration projects tend to eliminate riparian trees, thus the major effect of restoration on ecosystem function is warmer, more well lit streams that have higher algal production and higher nutrient uptake than their urban counterparts.

Related work in these same stream reaches by PhD student Christy Violin has found that macroinvertebrate community composition is quite different between the urban and forested streams (with many more sensitive taxa found in the reference streams), but that macroinvertebrate communities in the restored stream reaches are not different from their urban degraded stream counterparts. We have found that simple measures of habitat heterogeneity are the best predictors of macroinvertebrate community composition, and suggest the lack of attention to creating fine scale habitat diversity in restored streams may limit their success.

RECOMMENDATIONS

Our study to date has found that:

1) Streams in urban catchments have:
- Flashier hydrographs
- More highly incised stream channels
- Higher loads of both nitrate and total nitrogen (as well as Cl^- and $SO_4^=$)
- Simplified flow and substrate defined habitats
- Less variable distributions of organic material
- Very low occurrences of sensitive macroinvertebrate taxa

2) Restored streams differ from their urban degraded counterparts by
- Having less incised stream channels
- Having higher summer uptake efficiencies for NO_3^-
- Having reduced canopy cover relative to unrestored urban streams targeted for restoration

3) Restored streams are indistinguishable from their urban degraded counterparts in
- Having little variation of bed and flow habitat types
- Having low variation in depth and velocity
- Having nitrogen concentrations that are higher than reference watershed streams
- Having identical macroinvertebrate community composition

These findings suggest that restoration efforts are failing to ameliorate many of the insults to urban stream ecosystems. We recommend that increasing attention be paid to reestablishing fine-scale variation in habitat heterogeneity (introducing a variety of substrate sizes and varying depths within restored stream reaches) in order to better mimic less impacted streams. We caution that all urban restoration efforts are unlikely to succeed without addressing the primary cause of channel degradation, the flash hydrographs associated with high watershed impervious cover. Restoration of channels without accompanying stormwater management efforts are unlikely to be successful at reaching the goals of “reestablishing ecosystem function”.

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Improved Water Management Strategies for the Neuse basin Utilizing Climate−Information based Probabilistic Streamflow Forecasts

Basic Information

Title:	Improved Water Management Strategies for the Neuse basin Utilizing Climate−Information based Probabilistic Streamflow Forecasts
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Focus Category:	Water Quantity, Floods, Drought
Descriptors:	hydroclimatology, hydrological forecasting, watershed management, risk analysis, climate, decision models, flood control, multi−objective planning, optimization, reservoir modeling, systems analysis
Principal Investigators:	Sankarasubramanian Arumugam

Publication

1. Sankarasubramanian, A., N. Deveneni, and S. Ghosh, 2006, Multi−model Ensembling of Probabilistic Streamflow Forecasts: Role of Predictor State, Space in Skill Evaluation, Institute of Statistics Mimeo Series 2595,NC State University.
2. Arumugam, S., N. Devineni, and S. Ghosh, December 11−15, 2006. Multi−model Ensembling based on Predictor State Space: Seasonal Streamflow Forecasts and Causal Relations, American Geophysical Union Conference, San Francisco.
3. Devineni, N., A. Sankarasubramanian, S.Ghosh (2008), Multi−model Ensembling of Probabilistic Streamflow Forecasts: Role of Predictor State Space in Skill Evaluation, Water Resources Research, in press.
4. Golembesky, K., A.Sankrasubramanian, and N.Devineni, Improved Management of Falls Lake Reservoir during Summer Season Using Climate Information Based Monthly Streamflow Forecasts: Role of Restrictions in Water Supply and Water Quality Management, Under Revision in Journal of Water Resources Planning and Management, 2008.
5. Devineni, N., 2007, Multimodel ensembles of streamflow forecasts: Role of predictor state in developing optimal combination, M.S. Thesis, Department of Civil, Construction and Environmental Engineering, College of Engineering, North Carolina State University, Raleigh, NC 27695−708, 68 pages.
6. Arumugam, S. and N. Devineni, Predictability of U.S. Winter Precipitation: Role of ENSO state in Developing Multimodel Combinations, American Geophysical Union, Fall Conference, San Francisco, Dec 10−14, 2007.
7. Devineni,N., # and S. Arumugam, Multimodel Ensembles of Streamflow Forecasts: Role of Predictor State in Developing Optimal Combinations, American Geophysical Union, Fall Conference, San

Francisco, Dec 10−14, 2007. (#− Awarded Outstanding Student Paper Award for Devineni in Fall−2007 AGU conference).
8. Devineni, N., S.Arumugam, and S.Ghosh, Multi−model Ensembling based on Predictor State Space: Seasonal Streamflow Forecasts and Causal Relations, WRRI Annual Conference, March 27−28, 2007.
9. Golembesky, K., S.Arumugam, and N.Devineni, Improved Management of Falls Lake Reservoir during Summer Season Using Climate Information Based Monthly Streamflow Forecasts: Role of Restrictions in Water Supply and Water Quality Management, WRRI Conference, 2007.

Title

Improved Water Management Strategies for the Neuse basin utilizing Climate-Information based Probabilistic Streamflow Forecasts

Project Summary

A strategy to improve water allocation in the Neuse basin is proposed by developing a seamless integration climate-information based streamflow forecasts into water systems planning (3 months to 6 months) and operation. The proposed research will develop long-lead probabilistic streamflow forecasts in the Neuse basin contingent on both local land-surface and exogenous climatic conditions. Retrospective streamflow forecasts will be combined with a reservoir management model to understand the utility of streamflow forecasts in operating the Falls Dam. With the decadal variability in the tropical Atlantic Sea Surface Temperature (above-normal conditions) resulting in more hurricanes, it is imperative to develop a prognostic approach for water management in the Neuse basin given the basin accounts for 22% of state's population. Such an approach based on climate information could help water managers to prepare well in advance to reduce the impacts resulting from hydroclimatic extremes.

Benefits/Information from this project will support other ongoing activities in the Neuse including Neuse river basin planning program (supported by DENR), National Water Quality Assessment Program (supported by USGS) and NC Drought Monitoring (supported by Division of water resources, DENR) in coordination with the state's climate office. Analyses from this research will also promote identification of alternate river basin management plans during critical drought conditions including conjunctive use, instream flow maintenance and estuaries management. Informative web portal will de developed that summarizes the hydroclimatic predictability of the Neuse basin as well as updates of streamflow potential for the seasons ahead. Potential implications and its relevance to several ongoing researches in the Neuse basin will include quantitative representation of uncertainty in streamflows to support TMDL process, development of seasonal water management plans considering conjunctive use for the coastal zone and prediction outlooks for floods and droughts. We envisage that this effort for Neuse basin will motivate other basins in NC to incorporate to follow a prognostic, climate-information driven approach towards water management.

Methodology

Probabilistic Streamflow Forecasts Development: First, an assessment of different AGCMs' ability to predict both winter (January-March) and summer (June-September) precipitation over the Neuse basin will be investigated. This will be analyzed online at IRI data library (http://iridl.ldeo.columbia.edu/). Based on that, the best AGCM will be selected for developing the streamflow forecasting model. Three different approaches can be adopted when developing climate-information based streamflow forecasts: (a) Couple AGCM outputs with a Regional Spectral Model (RSM) whose outputs are combined with a large-scale watershed model16(b) Statistically downscale AGCM precipitation to streamflow at a particular point of interest18,19 (c) Develop a low dimensional statistical model that predicts the streamflow based on dominant climate predictors that influence the streamflow/rainfall potential over the basin17. Given only one year for this study, we will pursue approaches (b) and (c) to develop climate-information based streamflow forecasts. Coupling of AGCM with RSM and a watershed model will be pursued as future research activities. To pursue approach (b), we will develop different statistical downscaling methodologies to predict streamflow based on the selected AGCM's precipitation grids18,19. To develop a low-dimensional statistical model, detailed diagnostic analyses will be first carried out to identify the dominant predictors that influence the streamflow potential of the Neuse basin. The study will exploit the NC state climatological office's database (http://www.nc-climate.ncsu.edu/cronos/) and various other databases including climate Diagnostic Center (http://www.cdc.noaa.gov/PublicData/) to perform diagnostic analyses for predictor identification. We will employ state-of-the-art multivariate techniques including independent component analyses (ICA)20 to develop predictors that are independent to each other. Once the predictor set is developed, we will develop retrospective probabilistic streamflow forecasts for the Falls Lake using different statistical approaches including parametric and nonparametric regression techniques. Figure 6 shows an example of a probabilistic streamflow forecasts developed for a reservoir system in Ceara, North East Brazil.

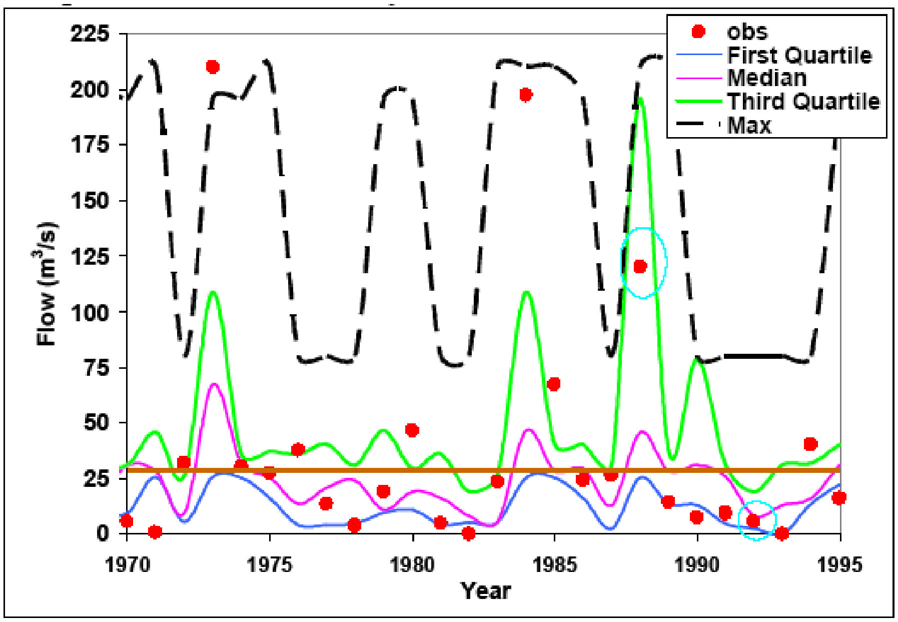

Figure 6: Retrospective, Leave one-out cross-validated 7-months ahead streamflow forecast ensembles for the Oros reservoir, Ceara, NE brazil17.The correlation between the observed streamflow and the predicted median streamflow is 0.7 over the period 1949-1996. Predictors employed for this purpose include Nino3.4 and Atlantic Dipole17. Note the ensembles shift according to the nature of flows for the two circled years in comparison to the long-term average (solid brown line). More than 60% of the mass is above the long-term average for the above normal inflow year in 1988, whereas in a below normal year in 1992, more than 80% of the mass is below the long-term average.

Reservoir Analyses: To develop a customized reservoir management model for Falls Lake, the study will employ MORAPS, which has been tested on many basins for climate forecasts application. Figure 7 shows a snapshot of MORAPS for representing a multi-reservoir system in the Greater Horn of Africa14. MORAPS incorporates novel features with the ability to run both retrospective analyses and to perform adaptive analyses of reservoir systems for real-time decision-making. Downscaled streamflow forecasts based on AGCM precipitation can also be used as an input to the model. MORAPS also incorporates a novel contract structure11,15 with an ability to perform the analyses under both optimization and simulation modes. Using retrospective streamflow forecasts developed from the study, we will employ MORAPS to identify optimal operating policies for the Falls Dam for reducing downstream flood damages as well as meeting both water quality and water quantity targets.

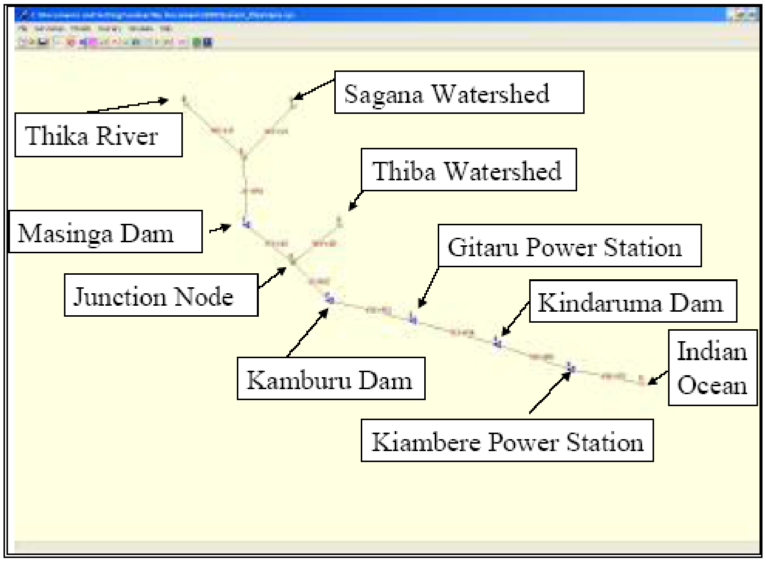

Figure 7: A snap shot of the Tana River 7-Forks Reservoir Management Model in the Greater Horn of Africa shown within MORAPS (Authors: Sankarasubramanian Arumugam, PI of this proposal and Upmanu Lall; copyrighted to IRI, Columbia University).

Dissemination: An important goal of this research is to develop a prognostic approach to improvise water management in the Neuse basin, which can help local/state water managers prepare well in advance for mitigation of the impacts resulting from hydroclimatic extremes. In this regard, we would like to invite NC DENR (Division of Water Resources), USACE (Wilmington District) and NC Drought Monitoring Council as external advisory board. In collaboration with them, we plan to perform the retrospective analyses of Falls Lake management contingent on probabilistic streamflow forecasts. A web portal will also be developed as part of the project that will update the long-lead streamflow forecasts for the Neuse basin on a monthly basis. We envisage that this effort for Neuse basin will motivate other basins in NC to incorporate to follow a prognostic, climate-information driven approach towards water management.

Relevance to Other Neuse Basin Initiative and Scope for Future Research

Given the diversity of hydrologic and geographic settings as well as water management issues related to the ongoing developments in the basin, Neuse has been a target basin towards monitoring, understanding and modeling of hydrological processes from both national and state agencies. Neuse which is located in the Albemarle-Pamlico drainage basin has been identified as one of the 20 selected basins for National Water Quality Assessment Program from the USGS33. Consortium of Universities for the Advancement of Hydrologic Science (CUAHSI), a collection of Universities for advancing hydrologic science, has identified Neuse as a target basin for setting up hydrologic observatory34. NC state DENR has developed a detailed water management plans to protect water quality and quantity issues in the basin given 22% of state's population live in the Neuse basin35,36,37. Several non-profit organizations, NC Water Resources Research Institute and Neuse River Foundation support many basin wide initiatives38,39,40. Research findings from this proposal will support the above programs in quantifying the uncertainty related seasonal streamflow potential as well as in providing prediction outlooks on floods and droughts. Findings from this research will also support future research initiatives of the PI that includes understanding the linkages between climate variability and ground water availability, utility of climate forecasts in reservoir/lake water quality management to guide the TMDL process and in understanding the importance of policy instruments in forecast applications in water management.

Principle Findings

Three specific objectives were proposed and the progress and the works needs to be completed under each objectives are detailed below.

(1) Development of a climate-information based streamflow forecasting model

Substantial progress has been made in streamflow forecasting model development. Two streamflow forecasting models were developed, one for January-March and another one for July-September, using Sea Surface temperature conditions. Both models have been verified and validated. Based on the comments on one of the reviewer in the proposal, we developed multimodel ensembles of streamflow forecasts and it has been found to improve single model forecasts. This work has been published as a report in the Institute of Statistics, Mimeo Series (http://www.stat.ncsu.edu/library/mimeo.html). We reviewed positive comments on the paper submitted to Water Resources Research and the revised version will be submitted soon.
We have also extended the multimodel combination methodology to combine various General Circulation Models (GCMs) so that multimodel precipitation forecasts could be used for downscaling to Falls Lake. Three different GCMs, ECHAM4.5, CCmV6, and COLA, were combined for the entire US. The multimodel precipitation forecasts are much better than the single model forecasts as shown below using Rank Probability Skill Score. Figure 1 shows the improvements that we obtained in predicting the US winter precipitation using the multimodel algorithm developed in this study. This is another paper in preparation for submission to the Journal of Climate.

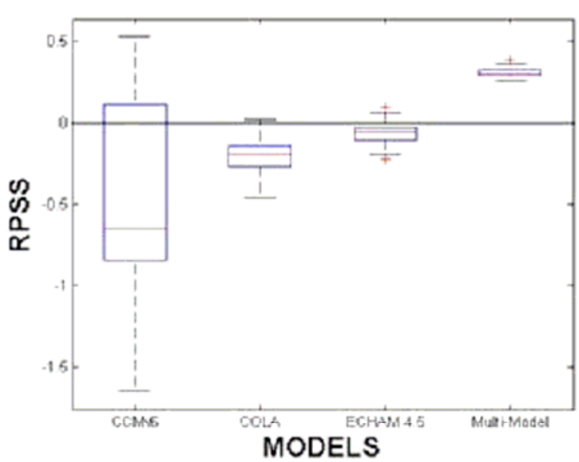

Figure 1: Improved Winter Precipitation Prediction for the U.S. using the multimodel combination algorithm developed in the study. The skill (given in terms of RPSS) of three different General Circulation Models - CCMV6 (Community Climate Model from UCAR) , COLA (University of Maryland), ECHAM4.5 (Max Planck Institute, Germany) – and multimodel precipitation forecasts in predicting the U.S. Winter Precipitation (December-

January-February). Note the improvements obtained from multimodel forecasts.

(2) Perform retrospective analyses on the utility of climate forecasts in improving Falls Lake operation

A good progress has been on meeting this objective, which is summarized as follows:
A Falls Lake Management Model has been developed and verified in modeling releases for the period July-August-September. The model has been tested with multi-model forecasts developed from the earlier objective and the importance of multimodel forecasts in improving reservoir management has been analyzed.

Figure 2 gives the probability of not meeting the target storage at 251.5 msl for the falls lake reservoir for individual model (resampling), climatology (no forecast) and multimodel. The secondary Y axis gives the observed flows. One can see very clearly that if the observed flows in a year is dry (below 0.33 percentile), and then the probability of not meeting the target storage is higher than that of climatology. Similarly, during a wet year (above 0.33 percentile), the probability of not meeting the target storage is lower than that of climatology. This is true for both multimodel forecasts and single model forecasts in all the years except 2005.
From figure 2, we can clearly see that the observed flow is dry, but the probability of not meeting the target storage given by resampling model is lower than that of climatology indicating it is a wet year. This is a false alarm. But, the prediction from the single model could be improved by using multimodel ensembles developed based on the predictor conditions. We see that the multimodel forecasts indicate clearly that the probability of not meeting the target storage is higher than that of climatology indicating correctly the flow is going to be dry. This is a fundamental contribution from this project. This paper will be submitted for possible publication in the Journal of American Water Resources Association.

Objective 3: Dissemination of results from the analyses to various state agencies that coordinate water-related activities in the Neuse basin.

A reasonable progress has been made and a project website has been developed with a content management system http://www.ce.ncsu.edu/research/hydroclimatology/. We issued a forecast for the summer of 2006 and it was reasonably on target (observed streamflow – 331 cfs and predicted streamflow – 346 cfs).

We are planning to issue a forecast for this year too. We will report our results in the web page.

<u>Significance</u>

Benefits/Information from this project will support other ongoing activities in the Neuse including Neuse river basin planning program (supported by DENR), National Water Quality Assessment Program (supported by USGS) and NC Drought Monitoring (supported by Division of water resources, DENR) in coordination with the state's climate office. Analyses from this research will also promote identification of alternate river basin management plans during critical drought conditions including conjunctive use, instream flow maintenance and estuaries management. Informative web portal will de developed that summarizes the hydroclimatic predictability of the Neuse basin as well as updates of streamflow potential for the seasons ahead. Potential implications and its relevance to several ongoing researches in the Neuse basin will include quantitative representation of uncertainty in streamflows to support TMDL process, development of seasonal water management plans considering conjunctive use for the coastal zone and prediction outlooks for floods and droughts. We envisage that this effort for Neuse basin will motivate other basins in NC to incorporate to follow a prognostic, climate-information driven approach towards water management.

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Protecting Receiving Waters: Removal of Biochemically Active Compounds from Wastewater by Sequential Photochemical and Biological Oxidation Processes

Basic Information

Title:	Protecting Receiving Waters: Removal of Biochemically Active Compounds from Wastewater by Sequential Photochemical and Biological Oxidation Processes
Project Number:	2007NC70B
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End Date:	8/31/2008
Funding Source:	104B
Congressional District:	2
Research Category:	Biological Sciences
Focus Category:	Treatment, Acid Deposition, Water Quality
Descriptors:	
Principal Investigators:	Detlef Knappe

Publication

Title: Protecting Receiving Waters: Removal of Biochemically Active Compounds from Wastewater by Sequential Photochemical and Biological Oxidation Processes

Statement of the Critical Regional and State Water Problem

The presence of biochemically active compounds (BACs) such as endocrine disrupting chemicals (EDCs), antimicrobial compounds, and other pharmaceutically active compounds (PhACs) in the aquatic environment is an issue of great importance. For example, the presence of EDCs may cause intersexuality in fish, and the presence of antimicrobial compounds may lead to the evolution of antibiotic-resistant bacteria. The Washington Post [1] recently reported that a USGS study found that at least 80% of male smallmouth bass caught in Virginia and Maryland tributaries of the Potomac River grew eggs. In addition, 54% of male largemouth bass caught in the Potomac River near the Blue Plains wastewater treatment plant (WWTP) of Washington D.C. showed signs of feminization and 23% were intersex.

While the specific cause of the feminization of male fish in the Potomac watershed has not yet been identified, other studies have linked incidents of intersexuality to the presence of EDCs that enter streams through WWTP discharges [2, 3, 4]. BACs are poorly removed during conventional wastewater treatment [5, 6, 7], and WWTP discharges are therefore an important source, through which BACs are introduced into the environment. Recent studies have shown that BACs are now ubiquitous in surface waters throughout the United States [8].

One can argue that similarities exist between the Potomac watershed and the Neuse River watershed in North Carolina. For example, rainfall, water usage, and population growth patterns are somewhat similar. Furthermore, the Potomac flows into the ecologically sensitive Chesapeake Bay, which serves as a breading and rearing ground for many species of fish in the Atlantic Ocean. In North Carolina, the Neuse flows into the similarly sensitive Pamlico Sound, and its watershed includes rapidly expanding urban areas (Durham, Cary, Raleigh). Conventionally treated wastewater from these municipalities is discharged into the Neuse or its tributaries. In addition, some water reuse projects have been implemented or are in the planning stage (irrigation, stream augmentation), which may also affect the water quality of the Neuse and its tributaries. Advanced wastewater treatment strategies are therefore needed that effectively eliminate the discharge of BACs into the environment.

Statement of Benefits

The goal of the proposed research is to develop an advanced wastewater treatment strategy that provides a barrier against the release of BACs into North Carolina surface waters and that yields readily biodegradable oxidation intermediates. Benefits of the proposed research include not only improved habitat for aquatic life, but also improved water quality for drinking water treatment plants that rely on surface water sources impacted by upstream WWTP discharges. The initial step of the proposed treatment strategy relies on the UV/H_2O_2 process to remove BACs via photolysis and hydroxyl radical oxidation. While this treatment option is expected to be effective for parent compound removal, little is known to date about the biochemical activity and environmental persistence of oxidation intermediates.

While *in vitro* biological activity assays such as the Yeast Estrogen Screen (YES) and Minimum Inhibitory Concentration (MIC) assay have been used to assess oxidative removal of estrogenic and antimicrobial activity, respectively, the results from such tests do not always concur with those obtained by *in vivo* tests. This point was illustrated in a UV/H_2O_2 treatment study [9] that showed that removal rates of estrogenic activity were more rapid when assessed by the YES assay than when assessed by an *in vivo* vitellogenin assay involving Japanese medeka fish. The results of the *in vivo* test suggested that some photooxidation intermediates exhibited estrogenic activity. Furthermore, little is known about the environmental persistence of such photooxidation intermediates. As a result, the proposed research will investigate what photooxidation conditions are required to produce BAC intermediates that are readily biodegradable. The biodegradation of photooxidation intermediates can be accomplished by microorganisms in (1) receiving streams (conventional WWTP discharge scenario or stream augmentation scenario), (2) biological filters that follow the photooxidation step, or (3) soils (e.g., water reuse scenarios involving irrigation or soil aquifer treatment). For the current proposal, scenario 1 will be simulated in Task 3. Scenarios 2 and 3 could be tested if follow-up funding can be obtained.

Nature, Scope, and Objectives of Research

Hypothesis: The hypothesis that will be tested in this research is that UV/H_2O_2 oxidation effectively removes BACs and their associated biological activity in WWTP effluent matrices. In addition, we will test the hypothesis that BAC oxidation products are readily biodegradable by microorganisms in NC surface waters. The validity of the above hypotheses will be tested with six BACs: the antimicrobial compounds sulfamethazine (SMZ), sulfadiazine (SDX), and trimethoprim (TMP), the EDCs bisphenol-A (BPA) and 17-α-ethinyl estradiol (EE2), and the analgesic compound diclofenac. Figure 1 depicts the chemical structures of the six contaminant probes as well as the position of the ^{14}C label (where applicable).

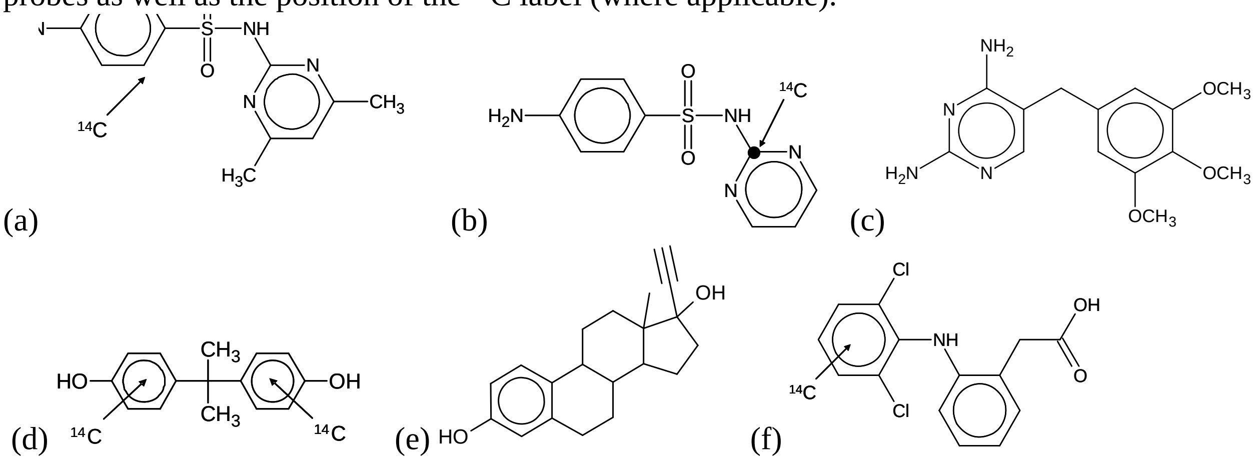


Figure 1: Chemical structures of (a) ^{14}C-sulfamethazine, (b) ^{14}C-sulfadiazine, (c) trimethoprim, (d) ^{14}C-bisphenol-A, (e) 17-α-ethinyl estradiol, and (f) ^{14}C-diclofenac.

These compounds were chosen for several reasons. First, many of these compounds are poorly degraded in WWTPs, and as a result have been detected in surface waters throughout the United States. Second, our group has experience with analytical methods for these compounds as well as with *in vitro* biological activity assays including the Yeast Estrogen Screen (YES) and Minimum Inhibitory Concentration (MIC) assays for detection of estrogenic and antimicrobial activities. Third, four of the selected BACs (SMZ, SDZ, bisphenol-A and diclofenac) are available in ^{14}C-labeled form, which will allow us to trace the biological mineralization of photooxidation intermediates ***at environmentally relevant concentrations***. Finally, SMZ and SDZ were specifically chosen because of the location of the ^{14}C label – tests with SMZ will allow us to test mineralization of the aniline moiety of sulfonamides while tests with SDZ will allow us to test the mineralization of the diazine moiety that is common in many sulfonamides.

Objectives: The principal objective of the proposed research is to quantify the effectiveness of combining UV/H_2O_2 and biological oxidation processes for the mineralization of six BACs (the antimicrobial compounds sulfamethazine, sulfadiazine, trimethoprim, the EDCs bisphenol-A and 17-α ethinyl estradiol, and the analgesic diclofenac) that commonly occur in conventionally treated wastewater. Specific objectives are (1) to evaluate the effects of the WWTP effluent matrix on photolysis and photooxidation rates of six BACs (**Task 1**), (2) to quantify the mineralization potential of ^{14}C-labeled BAC oxidation products as a function of UV/H_2O_2 oxidation conditions (**Task 2**), and (3) to measure biodegradation rates of ^{14}C-labeled BAC oxidation products by microorganisms in NC surface waters at different dilution rates (**Task 3**).

As part of task 1 will also quantify (1) effects of the WWTP matrix on removal rates of biological activity by YES and MIC assays and (2) effects of UV/H_2O_2 treatment conditions (UV fluence, H_2O_2 concentration) on effluent organic matter characteristic such as biochemical oxygen demand (BOD_5), assimilable organic carbon (AOC) concentration, dissolved organic carbon (DOC) concentration, and UV absorbance at 254 nm (UV_{254}).

Timeline. Figure 2 summarizes the timeline for the 3 research tasks, which are described in more detail below. Progress reports will be submitted on a quarterly basis, and I final report will be submitted by 2/29/08.

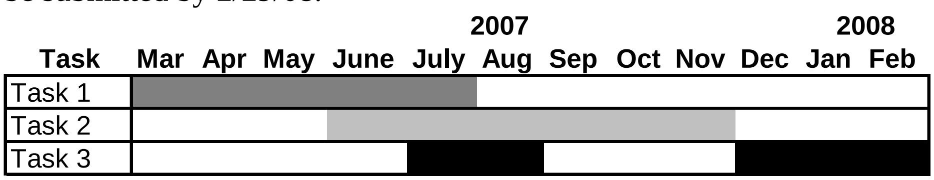


Figure 2: Timeline of project tasks.

Methods, Procedures, and Facilities

Task 1: Characterization of the photochemical oxidation process

Objectives: (1) Quantify and compare photolysis and UV/H_2O_2 photooxidation rates of six BACs (Figure 1) in ultrapure water and a WWTP effluent, (2) quantify removal rates of biological activity (estrogenic and antimicrobial activity), and (3) quantify changes in effluent organic matter (EfOM) characteristics as a result of UV/H_2O_2 treatment.

Approach: Photolysis and UV/H_2O_2 oxidation rates of BACs will be quantified with a quasi-collimated beam (QCB) apparatus, a schematic of which is shown in Figure 3. The purpose of the QCB apparatus is to ensure that the UV rays reaching the sample are exactly perpendicular, which allows for accurate measurement of UV energy at the surface of the sample. Therefore, we can accurately measure the UV fluence delivered to the sample. The QCB is equipped with a low pressure (LP) UV lamp, and LP UV fluences (doses) in the range of 0 to 1000 mJ cm^{-2} will be tested. Initial experiments will be conducted to quantify photolysis rates (no H_2O_2 added) of the six BACs shown in Figure 1. Experiments will be conducted in ultrapure water (UPW) and in WWTP effluent from the North Cary Water Reclamation facility (Cary, NC). These experiments will show whether the WWTP effluent matrix decreases (lower transmittance of UV light) or enhances (through sensitized photoprocesses that lead to the formation of reactive species in the WWTP effluent) BAC removal rates.

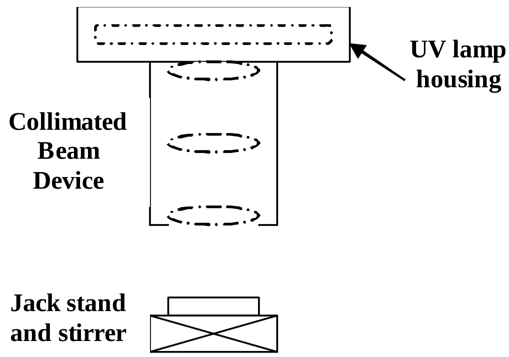


Figure 3: Schematic of basic "Quasi-Collimated Beam" UV Reactor

To quantify BAC oxidation rates in the UV/H_2O_2 process, additional QCB experiments will be conducted with the same UV fluence values used in photolysis experiments and with H_2O_2 concentrations of 2, 6, and 10 mg L^{-1}. Initial UV/H_2O_2 experiments will be conducted with the hydroxyl radical probe *para*-chlorobenzoic acid (*p*-CBA) to measure OH radical concentrations in UPW and in WWTP effluent [10, 11]. Once the OH radical concentration has been measured for each H_2O_2 concentration and water, removal rates of the 6 BACs will be predicted from photolysis rates (determined in the initial phase of this task), the measured OH radical concentration from *p*-CBA experiments, and second-order OH radical rate constants ($k_{OH,C}$) reported in the literature (Table 1). The method to be utilized for this prediction is outlined in detail in [11]. The predicted BAC removal rates will be verified with QCB experiments conducted in UPW and WWTP effluent spiked with individual BACs and with a mixture of all six BACs shown in Figure 1.

Table 1: Second-order rate constants for the reaction of OH radicals with selected BACs

Compound	$k_{OH,C}$ (M^{-1} s^{-1})	References
Sulfamethazine	$5x10^9$, $4.5x10^9$	12, 10
Sulfadiazine	$3.7x10^9$	12
Trimethoprim	$6.9x10^9$	13
Bisphenol-A	$1.02x10^{10}$	14
17-α-Ethinyl estradiol	$9.8x10^9$, $1.08x10^{10}$	14, 15
Diclofenac	$7.5x10^9$	15

BACs will be separated and quantified using HPLC methods already developed in our lab [10, 14].

Additional UV/H_2O_2 experiments will be conducted to measure removal rates of biological activity. The removal of estrogenic activity will be tested with BPA and EE2, spiked into WWTP effluent at environmentally relevant concentrations, and will be quantified with the YES assay [16]. For SMZ, SDZ, and TMP, the removal rate of antimicrobial activity will be measured with the MIC assay as outlined by [10].

Changes in EfOM characteristics resulting from UV/H_2O_2 treatment will be quantified by comparing BOD_5 levels, AOC concentrations, DOC concentrations, and UV_{254} values of the WWTP effluent prior to and following UV/H_2O_2 treatment. Data from earlier experiments will be used to select UV/H_2O_2 treatment conditions that lead to 90 and 99% removal of the most difficult to oxidize BAC. BOD, DOC, and UV_{254} will be measured using Standard Methods, and AOC will be measured using a new flow-cytometric method [17] that is and currently being implemented in our laboratory. Because of the quantities of water necessary to perform the

EfOM characterization tests, a 3-L recycle system equipped with an annular UV reactor containing a LP UV lamp (Trojan) will be used to conduct UV/H_2O_2 oxidation experiments for this portion of task 1. This reactor has been used previously for kinetic studies in our lab [10].

Task 2: Characterization of the mineralization potential of ^{14}C-labeled BAC photo-oxidation products

Objectives: (1) Using ^{14}C-labeled SMZ, SDZ, BPA, and diclofenac, quantify the mineralization potential of photooxidation intermediates in batch biological processes, and (2) determine UV/H_2O_2 treatment conditions for optimizing mineralization potential.

Approach: To achieve Task 2 objectives, batch reactors will be used to determine the mineralization potential of SMZ, SDZ, BPA, and diclofenac photooxidation intermediates. Batch tests permit screening of a wide range of photooxidation conditions and will be utilized to find optimal treatment conditions (UV fluence and H_2O_2 concentration) that maximize the conversion of BACs into readily biodegradable intermediates. In Task 2 experiments, WWTP effluent will be spiked with ^{14}C-labeled SMZ, SDZ, BPA, or diclofenac at environmentally relevant concentrations (~1 µg/L), and treated with a range of UV fluences (0-1000 mJ cm^{-2}) and H_2O_2 concentrations (0, 2, 6, and 10 mg L^{-1}). Upon photochemical oxidation, samples will be inoculated with a consortium of bacteria harvested from the Neuse River just upstream of the Raleigh WWTP. This consortium will be isolated and maintained as described by [17] and will permit sample inoculation with a known concentration of cells. The cell concentration of the sample will be determined by flow cytometry following staining with SYBRGreen [17]. SMZ and SDZ were chosen for this task because of the location of the ^{14}C label within their structure (Figure 1). By comparing mineralization rates of these two species, we can obtain information about the relative mineralization rates of photooxidation products derived from the aniline ring (SMZ) and the diazine ring (SDZ). Additionally, diclofenac is very recalcitrant in conventional wastewater treatment [18] and will thus be an excellent challenge BAC for testing the effects of UV/H_2O_2 treatment on enhancing mineralization potential.

Reactors for biological oxidation experiments were initially developed in our laboratory to test the bioavailability of sorbed organic contaminants (Figure 4, [19]). The reactors are equipped with a NaOH trap that will capture $^{14}CO_2$ produced from the mineralization of ^{14}C-labeled photooxidation intermediates. A second vial containing H_2O_2 will serve as a source of oxygen to maintain aerobic conditions.

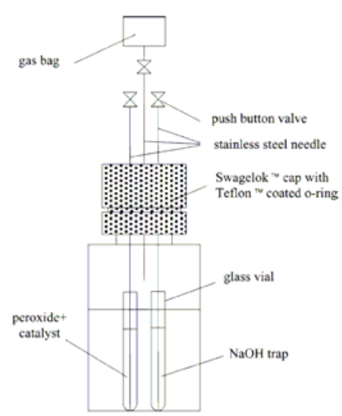


Figure 4: Batch reactor for biological oxidation experiments [19].

Contents of the NaOH trap will be periodically removed for analysis by liquid scintillation counting to determine the rate of $^{14}CO_2$ production, and replaced with fresh 2N NaOH solution. Batch tests will be terminated when $^{14}CO_2$ production is no longer measurable. At the completion of batch tests, ^{14}C in the aqueous phase (both suspended and dissolved) will be measured to obtain a ^{14}C mass balance. Results from experiments conducted without photooxidation pretreatment will serve as a baseline for assessing the effectiveness of photooxidation processes for enhancing biological mineralization. Control experiments, in which aerobic biological activity will be shut down via sodium azide addition, will be conducted in parallel with biotic experiments. All biological oxidation experiments will be conducted in triplicate.

Figure 5 displays a possible outcome of the biological oxidation experiments. Utilizing the same initial hydrogen peroxide concentration and varying applied UV fluence, Figure 5 depicts the expected cumulative formation of $^{14}CO_2$ as a function of time after inoculation of the photooxidized WWTP effluent. Increased UV fluence translates into a greater degree of BAC degradation and likely into the formation of more biodegradable products. The data of interest will be the initial rates of $^{14}CO_2$ formation and the mineralization potential, which is the maximum percentage of parent compound ^{14}C mineralized (visually represented as the plateau of Figure 5). The goal of Task 2 experiments is to identify the point of diminishing returns at which more severe UV/H_2O_2 oxidation conditions do not lead to enhanced mineralization rates and do not increase the mineralization potential during the biological oxidation step

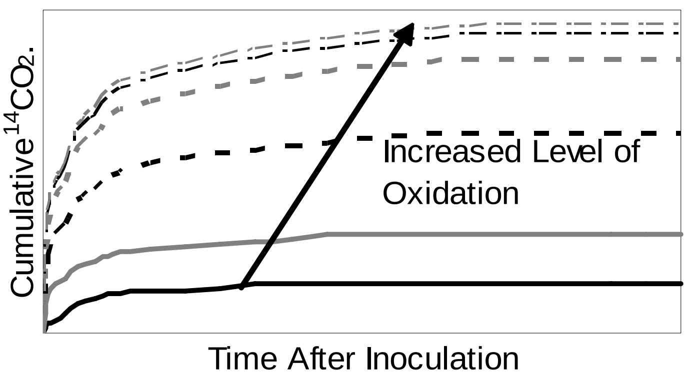


Figure 5: Potential outcomes from the Photooxidation and biodegradation experiments.

Task 3: Determination of mineralization rates of photooxidation products in receiving waters

Objective: Using one ^{14}C-labeled BAC, quantify the mineralization rate of photooxidation intermediates in batch biological processes designed to simulate the discharge of treated wastewater into a receiving stream.

Approach: Task 3 experiments are designed to simulate the discharge of photooxidized wastewater into receiving streams. The principal goal of the experiment is to quantify the mineralization rate of ^{14}C-labeled photooxidation intermediates in two NC surface waters at three dilution rates during warm and cold weather conditions. Task 3 experiments will be conducted with one ^{14}C-labeled BAC that proved to be the most challenging to remove in Task 1 and Task 2 experiments. In Task 3 experiments, WWTP effluent samples, spiked with the ^{14}C-labeled BAC, will first be treated with the optimal UV/H_2O_2 condition for maximizing mineralization potential as determined in Task 2. Upon photooxidation, samples will be mixed at levels of 10%, 50%, and 90% (v/v) with two NC surface waters: (1) Neuse River water (collected just upstream of the Raleigh WWTP) and (2) Pigeon House Branch water (collected at a proposed stream augmentation site). Photooxidized waters will be mixed with NC surface waters in the batch reactor to simulate variability in dilution rates that might occur when treated wastewater is discharged into small or large streams during wet, average, or drought periods. Furthermore, Task 3 experiments will be conducted with receiving water samples that will be collected during summer and winter months to investigate seasonal variations in mineralization rates of photooxidation intermediates. The water temperature of each stream will be recorded at the time of sampling, and the temperature conditions will be replicated during the biological oxidation experiments in our laboratory. Biological oxidation experiments will be initiated within one day of stream water sample collection.

Samples from the NaOH trap of the batch reactor (Figure 4) will be collected and analyzed as in task 2, and the experiment will be terminated when $^{14}CO_2$ production is no longer measurable. At the completion of batch tests, ^{14}C in the aqueous phase (both suspended and dissolved) will be measured to obtain a ^{14}C mass balance. Results from experiments conducted without photooxidation pretreatment will serve as a baseline for assessing the effectiveness of photooxidation processes for enhancing biological mineralization in receiving waters. Control experiments, in which aerobic biological activity will be shut down via sodium azide addition, will be conducted in parallel with biotic experiments. All tests will be conducted in triplicate.

Results from these experiments will yield a first approximation of the expected environmental half-lives of BAC photooxidation intermediates. This information will be useful to estimate the degree of mineralization that can be accomplished by stream microorganisms between a WWTP discharge location and a downstream water treatment plant intake. Also of interest will be an estimate of the expected mineralization that would occur in the Neuse River between a WWTP discharge and Pamlico Sound.

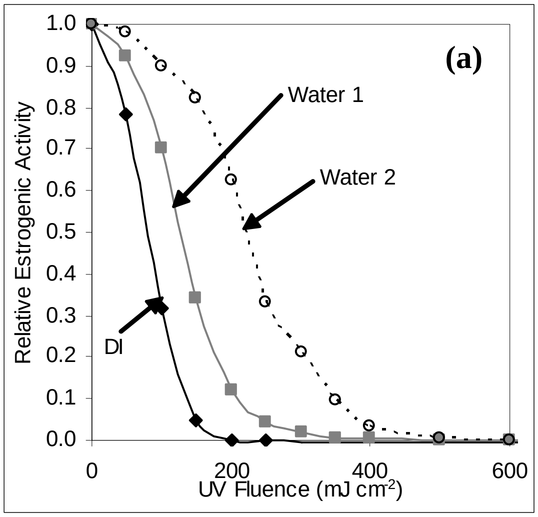
Facilities

Equipment required to conduct the proposed research (collimated beam apparatus, annular UV reactor, HPLC, liquid scintillation counter, incubators) is available in the Environmental Engineering research laboratory at NC State University or is available for our use on the NC State campus (flow cytometer).

Related Research

Since the first documented example of a widespread sexual disruption in wild fish resulting from exposure to ambient levels of BACs in British waters was reported in 1998 [20], many studies have detected BACs in surface waters throughout the world [e.g., 8, 21, 22]. One important source of BACs is the discharge pipe of municipal WWTPs, which do not effectively remove a number of BACs before discharge into receiving waters [23, 24].

The advanced oxidation technology proposed for use in this study is the UV/H_2O_2 process. In this process, hydrogen peroxide molecules absorb UV energy and break down into hydroxyl radicals ($\cdot OH$). The hydroxyl radical is highly reactive, oxidizing many BACs with rate constants on the order of $10^9 – 10^{10}$ M^{-1} s^{-1} [e.g. 13-15]. To date, most studies evaluating the effectiveness of the UV/H_2O_2 process for BAC removal and removal of associated biochemical activity have focused on drinking water treatment conditions and thus evaluated process performance in relatively clean water matrices ranging from pure lab water to stream and lake waters [e.g. 10, 13, 16]. These studies have shown that although UV/H_2O_2 oxidation is negatively impacted by the presence of background organic matter and high alkalinity, the process is still capable of oxidizing BACs and removing associated biological activity at reasonable operating conditions. For example, estrogenic activity associated with EE2 was almost completely removed using LP UV and 5 mg L^{-1} H_2O_2 with 200 mJ cm^{-2} applied UV fluence while 400-500 mJ cm^{-2} of applied fluence were required to achieve similar removals in two surface waters (Figure 6a) [14]. While UV disinfection typically occurs at fluence values of less than 100 mJ cm^{-2}, a full scale UV/H_2O_2 oxidation process at a Dutch drinking water treatment plant designed for pesticide removal utilizes UV fluence values on the order of ~600 mJ cm^{-2} [25].



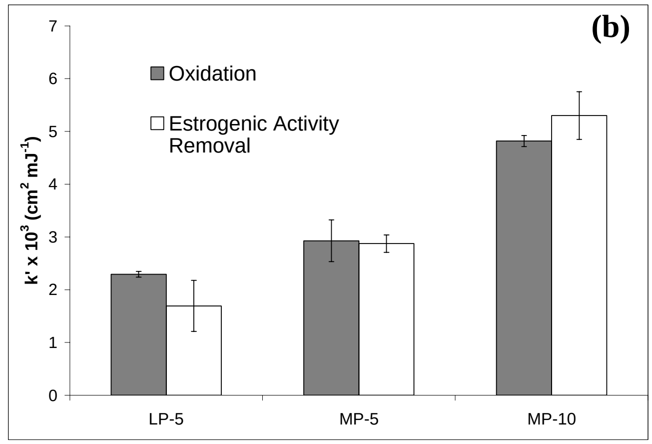


Figure 6: (a) UV/H_2O_2 removal of estrogenic activity in DI water and 2 natural waters and (b) comparison of EE2 oxidation and estrogenic activity removal rates with low pressure (LP) and medium pressure (MP) UV/H_2O_2 processes; value after LP or MP signifies H_2O_2 concentration in mg/L (from [14])

In some instances (e.g., EE2 (Figure 6b) [14] and sulfamethoxazole and SMZ [10]) removal rates of the parent BAC and the associated biochemical activity are identical; however, this lock-step behavior does not always occur. In [9], removal of estrogenic activity as measured with the *in vitro* YES assay was slower than oxidative removal of BPA. When an *in vivo* assay measuring vitellogenin induction in male fish was utilized to detect removal of estrogenic activity associated with BPA, the disparity was more pronounced. Additionally, an increase in larval mortality was observed at certain UV/H_2O_2 treatment conditions. The paper reiterates that although UV/H_2O_2 is effective in oxidizing BACs, the byproducts may still be harmful.

Water quality can be improved with UV/H_2O_2 treatment if we can show that BAC oxidation products are readily biodegradable at environmentally relevant concentrations. Oxidation processes yield intermediates that are more biodegradable than the parent compounds, and this concept is being exploited in the treatment of industrial wastewaters. By 2001, more than 100 literature examples were available indicating the plausibility and utility of sequential chemical and biological oxidation processes for the treatment of wastewaters containing recalcitrant pollutants such as effluents from textile mills, paper mills, tannery, and olive mills, pesticide wastewaters, etc. [26]. However, little is known to date about the fate of oxidation intermediates of organic compounds that are present at trace levels in municipal wastewaters. The proposed work will fill this gap, examining how photooxidized BAC intermediates are biodegraded by natural consortia of microorganisms.

Training Potential

It is anticipated that **one PhD student** (Carolina Baeza) will be trained in the field of Environmental Engineering by participating in this project. This student has been supported on an NSF Graduate Research Fellowship and is now on an NWRI fellowship that will continue to provide partial support throughout the course of the proposed project. In addition, **one visiting scholar** (Erik Rosenfeldt) will contribute to this project. The PI has worked and continues to involve one to two undergraduate research assistants in ongoing research project. It is anticipated, that **one undergraduate student** will be trained by the proposed research project.

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Curriculum Vitae

Detlef Knappe		Phone:	919-515-8791 (office)
Associate Professor			919-828-1548 (home)
Dept. of Civil, Construction, and Environmental Engrg.		Fax:	919-515-7908
North Carolina State University		E-mail:	knappe@eos.ncsu.edu

Education

Ph.D.	Environmental Engineering	University of Illinois, Urbana, IL	January 1996
M.S.	Environmental Engineering	University of Illinois, Urbana, IL	May 1991
B.S.	Civil Engineering	University of Illinois, Urbana, IL	May 1989

Professional Experience

2002 – present	Associate Professor, Dept. of Civil, Construction, and Environmental Engineering, NC State University
2005 (7/1-12/31)	Visiting Scholar, EAWAG, Swiss Federal Institute of Technology (ETH) Zürich, Switzerland
1996 - 2002	Assistant Professor, Department of Civil Engineering, NC State University

Research Interests

Trace organic contaminants, adsorption processes, advanced oxidation processes, coagulation, effects of algae on water quality and treatment processes, natural organic matter, bioavailability, fate of organic contaminants in landfills.

Teaching Interests

Physical/chemical fundamentals of environmental engineering processes, water treatment plant design, unit operations and water chemistry laboratories.

Recent Publications (Water-Treatment-Related)

1. Baeza, A.C. and D.R.U. Knappe. "Removal of Sulfonamides and Associated Antimicrobial Activity by UV Photolysis and UV/H_2O_2 Processes." In *Proc. 2006 AWWA Annual Conference and Exhibition*, San Antonio, TX, June 11-15, 2006.
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Selected Current Research Projects (Water-Treatment-Related)

1. Removal of antimicrobial compounds and their associated biochemical activity by UV/H_2O_2 processes. Student support through NSF Graduate Fellowship and NWRI Fellowship.
2. Removal of 2-methylisoborneol and geosmin with high-silica zeolites and zeolite-enhanced ozonation. PI: Knappe, D.R.U. Agency: AwwaRF ($150,000; 1/1/06 – 12/31/07).
3. Evaluation of Computational Fluid Dynamics (CFD) for Modeling UV-Initiated Advanced Oxidation Processes. PI: Ducoste, J.J., Co-PI Knappe, D.R.U. Agency: AwwaRF ($150,000; 1/1/06 – 12/31/07).
4. Predicting Single-Solute Adsorption Isotherms for Non-Regulated Contaminants from Fundamental Adsorbent and Adsorbate Properties, PI, U.S. EPA ($65,000; 8/16/03 – 9/30/06).

Professional Activities, Honors, and Awards

<u>Professional Society Memberships</u>:

American Society of Civil Engineers, American Water Works Association, International Water Association, American Chemical Society, American Geophysical Union, Association of Environmental Engineering and Science Professors.

<u>Registration</u>:

EIT, Illinois

<u>Honors and Awards:</u>

- 2006 AWWA Water Science and Research Division Best Poster Award for poster entitled "Removal of Sulfonamides and Associated Antimicrobial Activity by UV Photolysis and UV/H_2O_2 Processes"
- 2003 Bill Horn Kimley-Horn Faculty Award for excellence in graduate and undergraduate teaching and other accomplishments, NC State University
- 2001 AWWA Water Science and Research Division Best Paper Award
- 2000 Young Civil Engineer Achievement Award, University of Illinois.
- Thesis advisor of David Briley whose MS thesis entitled "Optimization of Coagulation Conditions for the Removal of Algae in Conventional Water Treatment" won 2nd place in the 1999 AEESP/Montgomery Watson Master's Thesis Award competition.

Stable Isotope Tracers to Quantify Impervious Area Effects on Baseflow to Coastal Plain Streams

Basic Information

Title:	Stable Isotope Tracers to Quantify Impervious Area Effects on Baseflow to Coastal Plain Streams
Project Number:	2007NC75B
Start Date:	3/1/2007
End Date:	2/29/2008
Funding Source:	104B
Congressional District:	1 &3
Research Category:	Ground−water Flow and Transport
Focus Category:	Groundwater, Hydrology, Surface Water
Descriptors:	
Principal Investigators:	Michael A ODriscoll, Mark M. Brinson

Publication

1. Hutchinson, H.W. 2007. Hydrologic and isotopic response of low−order Coastal Plain streams to urban land−use. Department of Geological Sciences (MS), East Carolina University. 126 p.
2. Soban, J.R. 2007. Department of Geological Sciences (MS), East Carolina University. Stream channel response to urban land use in the inner Coastal Plain of North Carolina. 154 p.
3. DeLoatch, J.P., O’Driscoll, M.A., Brinson, M.M., and Hardison, E.C. 2007. Groundwater hydrology of low−order Coastal Plain streams across an urban land−use gradient. Geological Society of America National Meeting, Denver, CO. Geological Society of America Abstracts with Programs, Vol. 39, No. 6, p. 319.
4. Hardison, E., O’Driscoll, M., Rheinhardt, R., and Brinson, M, 2007. Effects of urbanization on low−order riparian and stream systems in Coastal Plain North Carolina. Society of Wetland Scientists Annual Meeting 2007. June10−15, 2007. Sacramento, CA.
5. Hardison, E., Brinson, M, and O’Driscoll, M. 2007. A comparison of total versus effective impervious surface area for the prediction of water quality degradation in low−order coastal plain streams. North Carolina Academy of Sciences Annual Meeting 2007. March 31, 2007.
6. Hardison, E., Brinson, M, and O’Driscoll, M. 2007. Effects of Total and Effective Impervious Surfaces on Water Quality in Low−Order Coastal Plain Streams, North Carolina. ECU Research and Creative Achievement Symposium. March 26, 2007.
7. Soban, J. and O’Driscoll, M. 2007. Stream channel response to urban land−use in the North Carolina Coastal Plain. Geological Society of America Abstracts with Programs Vol. 39, No. 2. p.18. SE Section, Savannah, GA. March 29, 2007.
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(1) **Title**: Stable isotope tracers to quantify impervious area effects on baseflow to Coastal Plain streams.

(2) **Statement of Critical Regional or State Water Problem:**

Across the globe human populations are becoming increasingly urban. Current estimates suggest that fifty percent of the world's population lives in urban areas (Cohen 2003). In the southeastern United States urban land-use is rapidly expanding. Future projections indicate that over the next 40 years there will be a 3-4 fold growth in the amount of impervious area in the southeastern US. North Carolina is one of the most rapidly growing states in the southeast (6th fastest in U.S., 1990-2000). Statewide 18.1% of watershed area is affected by greater than 5% total impervious area. In the southeast only Florida has experienced more land-use alteration (31.2%). By 2030 projections indicate that 27% of North Carolina's watersheds will have >5% total impervious area (Exum et al. 2005). Urban land-use has been shown to alter and degrade streams by changing their hydrologic response to precipitation (McBride and Booth 2005). These findings suggest that streams in North Carolina are at significant risk for degradation due to current and future land-use changes. Stream degradation associated with increased imperviousness has been referred to as the "urban stream syndrome" and symptoms include: increases in overland flow and stormwater runoff, greater peak discharges, shortened lag times to peak flow, increased water temperature variability (Krause et al. 2004), shallow ground water quality degradation (Bruce and McMahon 1996), elevated nutrients and contaminants, altered biotic assemblages, reduced biotic richness, reduced channel complexity, increased dominance of tolerant species, and altered nutrient processing and ecosystem functioning (Meyer et al. 2005). Ground water recharge in urban areas is complex because normal recharge pathways are often altered. Alteration of natural recharge pathways in urban areas leads to changes in precipitation intake at the land surface. The literature suggests variable responses of baseflow to increased urban land-use (Walsh et al. 2005a). Baseflow magnitude may decrease if impervious area results in less ground water recharge and more stormwater runoff or if interbasin transfers occur. Channel incision related to stormwater runoff may increase drainage of near stream aquifers. Leaky underground pipes transporting sewage, stormwater and drinking water may provide recharge and increase baseflow (Lerner 2002).

Currently it is not known what threshold percentage of watershed impervious area will begin to affect stream baseflow. Different physiographic regions respond differently to increases in impervious cover and stormwater stresses. The relationship between impervious area and in-stream response must be established for the common physiographic settings in North Carolina: Mountains, Piedmont, and Coastal Plain. The effects that watershed impervious area has on baseflow to Coastal Plain streams will be quantified in this study. Quantification of impacts on Coastal Plain river baseflow is important because over 50% of annual average streamflow of these rivers is derived from baseflow (McMahon and Lloyd, 1995) and the Coastal Plain comprises 45% of the land area of North Carolina. Baseflow reductions in Coastal Plain streams could cause chemical contamination problems to worsen, increased water temperatures and lowered dissolved oxygen concentrations, alterations to fresh water fluxes to estuaries and the coastal ocean, and reduced buffering of climate change. Typically, stormwater management is dealt with on a site by site basis with no measurement of the cumulative effects on the entire watershed. The value of this proposed approach is that stable isotope measurements can reveal the cumulative effects of upstream land-use changes at the watershed outlet.

(3) **Statement of Results, Benefits and/or Information**

This proposal addresses the NC WRRI 2007-2008 priority research area D.3: **Determine the percent impervious surfaces in a watershed that would affect surface water recharge by ground water.** The major contribution of this research is that it will determine the threshold at which the percentage of watershed impervious area has measurable effects on baseflow contributions to Coastal Plain streams and evaluate the effectiveness of stable isotope techniques to quantify this threshold at the watershed-scale. This work will document variations in ground water inputs to streams across an urban gradient, and determine if stable isotope techniques are effective at quantifying the watershed-scale effects of impervious area and stormwater management. Stable isotope data will be compared with physical (discharge and water temperature) and chemical (specific conductance) measurements, to determine if lower-cost applications may also yield similar results.

The benefits of this study are two-fold. First, it will provide detailed information on the threshold levels of impervious area that result in observable changes in baseflow magnitude to Coastal Plain streams. Monitoring of the surface water-ground water interactions at 6 locations and over a range of streamflow conditions will characterize the changes in surface water-ground water interactions that occur across a gradient of impervious area. Second, it will provide a methodology for using stable isotopes to characterize the effects of watershed impervious area on stream hydrological response at the watershed scale. Such a methodology may prove to be applicable in a wide variety of river settings.

Important outcomes of this research will include:

(i) An improved understanding of the relationship between impervious area and stormwater/ baseflow response in Coastal Plain streams.

(ii) Determination of impact thresholds of impervious area, above which alterations in baseflow magnitude are measurable.

(iii) Evaluation of a stable isotope technique to characterize the effects of land-use on surface water-ground water interactions. It is expected that this methodology will be applicable in the Coastal Plain and other settings.

(iv) Comparison of stable isotope techniques with other practical approaches (physical hydrograph separation, seepage runs, specific conductance, water temperature, flashiness,) to quantify baseflow and stormwater responses to land-use alterations

(v) The improvements to our current monitoring network and continuation of data collection may result in future funding to create a long-term Coastal Plain urban stream monitoring network.

(vi)The urban stream monitoring network will be useful for labs and class field trips for hydrology-related courses.

(vii) Study results can provide valuable information for stormwater managers in Greenville and similar Coastal Plain cities. This study will also provide baseline conditions, which are useful for the evaluation of land-use effects on rivers in an area that is rapidly developing.

(4) **Nature, Scope, and Objectives of Research and Timeline of Activities**

The study goals are to determine thresholds of impervious area, above which alterations in baseflow magnitude are measurable and to evaluate a stable isotope approach to quantify the watershed-scale effects of stormwater runoff on Coastal Plain streams. The objectives of this study are to:

(i) Characterize seasonal variability of river-ground water interactions across an urban land-use gradient using stable isotope and physical measurements.

(ii) Quantify the variability in isotopic composition of stream runoff during storm events across an urban gradient.

(iii) Evaluate the effectiveness of stable isotope tracers for quantifying the hydrologic effects of impervious area on Coastal Plain streams. To determine if stable isotopes can effectively quantify the changes in hydrology due to land-use, at what level of accuracy this can be done, and at what percentage of watershed impervious area are differences in baseflow noticeable. Overall, to determine if stable isotope tracers can accurately measure the watershed-scale effects of stormwater runoff from impervious areas.

(iv) Compare a variety of hydraulic and geochemical measurements with isotopic results to determine if other indicators of stormwater/baseflow variations can provide similar results.

Precipitation, ground water, and surface water monitoring activities will support the research objectives by providing data that captures baseflow and runoff variability across an impervious area gradient, within storm events and across the seasons. The data will show the natural variability and the magnitude of differences in baseflow due to land-use change. In order to be confident in the integrity of stable isotope evaluation of land-use effects the margin of error for this technique must be determined by evaluation of monitoring data.

Schedule of Tasks

			2007						2008					
TASK	J	F	M	A	M	J	J	A	S	0	N	D	J	F
A.			---											
B.			---	---	---	---	---	---	---	---	---	---	---	---
C.			---	---	---	---	---	---	---	---	---	---	---	---
D.											---	---	---	---
E.											---	---	---	---

A. Add monitoring equipment to the monitoring network and begin precipitation, surface water, and ground water sampling
B. Storm event sampling (6 storms – 1-2/season)
C. Ground water and surface water monitoring
D. Analysis and synthesis of hydrologic, chemical, and isotopic data
E. Write final report and manuscript

(5) **Methods, Procedures, and Facilities**

General Methodology

The primary way that urbanization affects streams is by altering surface flow patterns. Stable isotope tracers may be used to quantify changes in stormwater runoff and ground water inputs to streams. ^{18}O and Deuterium (D) are naturally occurring stable isotopes that are present in rainfall, surface water, and ground water. Seasonal stable isotopic variations in precipitation have been used to study the movement and source of subsurface water in various settings (Kendall and McDonnell 1998). ^{18}O and D provide a seasonal meteoric signal in temperate, continental systems that is often attenuated in shallow ground water (Clark and Fritz, 1997). Rainwater tends to be depleted in winter and enriched in summer and ground water tends to have an average isotopic composition that fluctuates little throughout the year. For this reason, storm event precipitation isotopic composition is quite different from baseflow composition, allowing for two component storm hydrograph separations of baseflow or “old water” and runoff or “new water” (Sklash and Fritz 1975, Buttle 1994). In this study, the ^{18}O and D composition of precipitation, surface water, and ground water will be quantified seasonally and for storm events across an urban land-use gradient.

A field-based hydrologic and geochemical approach will be used to quantify the effects of increased impervious area on stream hydrology. The study area will consist of sub-watersheds of the Tar River from Greenville to Grimesland, North Carolina. The Tar River basin is a good location for evaluating the effects of urbanization on Coastal Plain streams; from 1982 through 1997, the major land use changes have been a 90.1% increase in urban land-use (NCDENR 2004). In 2005, an urban runoff monitoring network was set up in the vicinity of Greenville, NC to monitor the effects of land-use on Coastal Plain streams. Currently this monitoring network is being used for an Ecosystem Enhancement Program (NC-DENR) funded project to collect detailed data on urban riparian systems in the Coastal Plain. Six sites have been selected across an urban land-use gradient within the Tar River Basin, with watersheds ranging in size from 1.19 to 3.46 km^2 and impervious areas have been quantified ranging from 37% to less than 5% (Figure 1). Future work will aim to quantify the effective impervious area for these watersheds and how this relates to various hydrologic metrics. All of the sites are in similar geological settings and within 5 miles of each other and should be subject to similar meteorological conditions. Therefore hydrologic differences between sites are presumably due to land-use differences.

Preliminary data suggest that urban land-use has modified stream hydrology in the Greenville area, with direct relationships between degree of alteration and percentage of watershed impervious area. Stream discharge data and hydrograph separation modeling (Lim et al. 2005) suggests that baseflow decreases and storm runoff increases with increased watershed impervious area (Figure 2). Water temperature increases during summer storm events were also correlated to watershed impervious area (Figure 3). Stable isotope composition data for a June 2006 storm indicates that ^{18}O composition data for storm samples varies directly with watershed impervious area and that stable isotope tracers may be useful indicators of land-use effects on stream hydrology in this and other Coastal Plain settings (Figure 4). This proposed research will aim to evaluate how well this relationship holds up throughout the year and with storms of differing intensities.

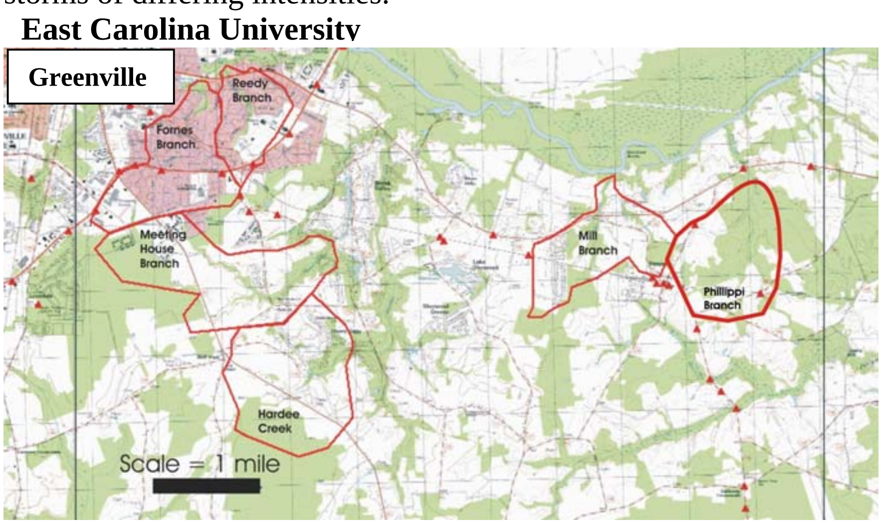

Figure 1. Location of watersheds within urban stream monitoring network, Greenville, NC.

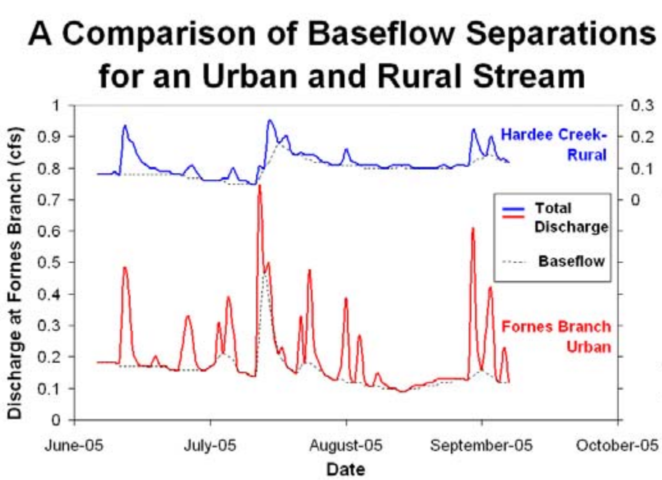

Figure 2. A comparison of baseflow separations for daily discharge during summer, 2005 for an urban stream (Fornes Branch, 37% impervious area) and a rural stream (Hardee Creek, 14% impervious area). For the summer of 2005 the urban stream had baseflow contributions of 77% whereas the rural stream had baseflow contributions of 85%. The urban stream had 8% greater stormwater runoff inputs.

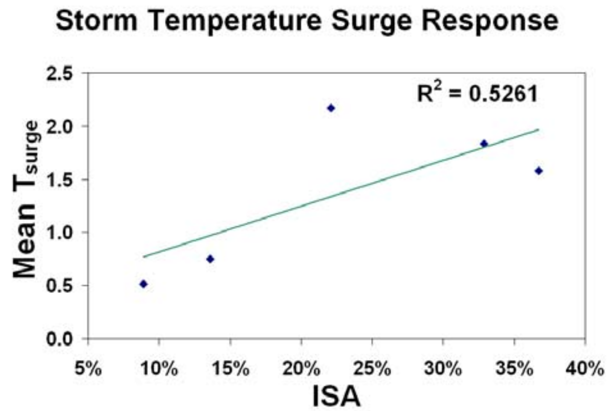

Figure 3. Stream temperature response across urban land-use gradient for storm events monitored during the summer of 2005. Temperatures are in degrees-C, n=23 storms, (ISA) impervious surface area.

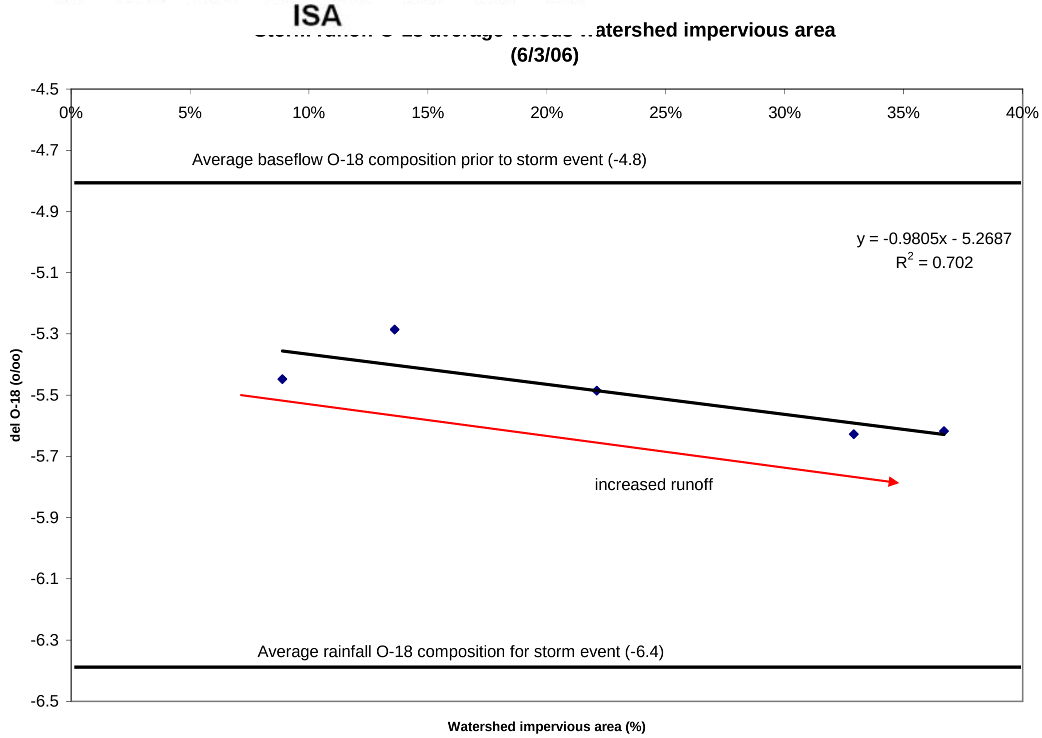

Figure 4. Stream ^{18}O response across an urban land-use gradient for a storm event sampled on June 3, 2006. Stormwater ^{18}O was sampled approximately 5 times during the storm event, baseflow ^{18}O was sampled prior to the event.

Hydrologic and Isotopic Monitoring

River water will be sampled at the six sites during storm flows for six storm events throughout the year. The goal is to obtain at least 5 samples during an event to provide a composite stormwater sample (2 rising limb, 1 peak, 2 falling limb samples). Automatic water samplers will be used for storm sampling. In addition, first flush sampling will be conducted using Nalgene© first flush single sample bottles installed at similar elevations at each site. The data collected from these will indicate if first flush sampling is as effective as composite sampling for evaluating land-use effects on hydrology. Prior to storm events a stream water sample will be collected to characterize baseflow (ground water) conditions. Water samples will also be collected from floodplain ground water wells adjacent to the streams. The ground water well samples will be compared with stream baseflow and runoff samples to determine if floodplain well water composition can provide evidence of the effects of upstream impervious area on floodplain hydrology. Cross-sections of each stream will be surveyed to determine if incision due to urbanization has effects on ground water inputs. Precipitation during storm events will be collected to capture the ^{18}O input signal. During periods between sampled storm events, baseflow and ground water well samples will be collected manually every 2-weeks. These data will be used to estimate mean residence time (Clark and Fritz 1997).

Precipitation for seasonal analysis will be collected on a bi-weekly basis in a buried bottle with a screened funnel and coiled tube. Oil will be added to prevent evaporation.

To prepare all water samples for lab analysis, water will be gravity filtered through a non-ashed carbon filter into 1 mL sample bottles. These samples will be analyzed at Duke Environmental Stable Isotope Laboratory (DEVIL) using a ThermoFinnigan Thermochemical element analyzer (TCEA) coupled to a ThermoFinnigan Delta-plus XL mass spectrometer. Isotopic composition values have a 0.1 to 0.2 per mil ^{18}O and 0.5 to 1.0 per mill Deuterium precision. Prior to analyzing all storm samples, a baseflow and a rainfall sample will be analyzed to ensure the two-components have different ^{18}O composition so the data can be analyzed using a 2-component mixing model (DeWalle and Pionke 1994) . To further ensure quality control, random blind duplicates will be sent in once for every month of baseflow and rain samples. Each storm sample set analyzed will also contain a blind duplicate. After sample analysis, a two-component stable isotope mixing model will be used to determine the amount of ground water feeding rivers during storm flows at each site and for each of the monitored storm events. The percentage of ground water input for all sites and events will be compared with urban land-use category to determine if the magnitude of ground water inputs is related to land-use.

Currently stage recorders and ground water level recorders are in operation at each site and stage-discharge rating curves have been developed to determine discharge variations across watersheds. This instrumentation will continue to be used to monitor surface water and ground water stage during the study period. In addition, water temperature recorders currently in use will continue to monitor surface and ground water temperatures during the study. A weather station is being operated to monitor rainfall amounts in Greenville and this monitoring will continue for the duration of the study. For this proposed study, additions to the monitoring network will include specific conductance loggers to monitor surface water specific conductance at each site. We will use specific conductance as a surrogate for Cl. The goal is to compare storm runoff and baseflow specific conductance data with stable isotope data to verify if specific conductance behaves conservatively and is a good metric to quantify the effects of land-use on hydrology in these settings. Preliminary data collected along Green's Mill Run suggests that specific conductance may be a good tracer of surface water-ground water interactions. Hydrograph separations performed using specific conductance will be compared with those performed using stable isotopes. Additional metrics that will be compared with stable isotope data include discharge and stage daily coefficient of variation, lag time, rising stage frequency (number of time periods when stage rises at least 0.1 ft), median duration of high stage (> 90 th percentile)(McMahon et al. 2003), flashiness: fraction of the year daily discharge> annual mean discharge (Booth et al. 2004), storm temperature surge, seepage runs (1 summer, 1 winter), and physical hydrograph separations (Lim et al. 2005).

<u>Major Research Tasks</u>

Task 1: Characterize the variations in stable isotopic composition of rainfall, surface water, and ground water and the hydrology of the sites.

Task 2: Quantify the effects of land-use and how it relates to the threshold at which baseflow is affected by impervious cover.

Task 3: Compare isotopic results with other indicators to evaluate how well they reflect land-use effects.

Isotopic Data	Chemical Data (Specific Conductance)	Physical Data
Storm	**Storm**	**Temperature**
First flush sample	First flush sample	Air temperature (1/2 hr)
Storm composite sample	Storm composite sample	Stream and gw temperature (1/2 hr)
Baseflow	**Baseflow / logger (1/2 hr)**	**Stage and discharge**
Bi-weekly sample	Bi-weekly sample	Surface water stage (1/2 hr)
Pre-storm sample	Pre-storm sample	Ground water stage (1/2 hr)

Table 1. Storm and baseflow monitoring parameters

(6) **Related Research**

Watershed impervious area has been related to stream degradation in many settings (Shuster et al. 2005). Based on a review of the literature, Paul and Meyer suggest that many thresholds of stream degradation are associated with an impervious surface cover of 10-20% (2001). Little is known about the threshold at which impervious area affects baseflow to streams. Recently Brandes et al. (2005) found that baseflow response to urbanization was not consistent across three physiographic regions of the Lower Delaware River (Valley and Ridge, Piedmont, and Coastal Plain provinces). They looked at 10 watersheds (six urban and four reference) and found that only one (the Cooper River in the Coastal Plain) showed declines in baseflow over time. The decrease in baseflow was in part related to the impervious area of 21 %, but also likely due to interbasin transfers. Recent work by Brinson et al. (2006) indicates that land-use changes, specifically channelization associated with agriculture, has resulted in an alteration of groundwater flows to many low-order Coastal Plain streams. It is unknown what threshold of impervious area is likely to affect baseflow to rivers in the Coastal Plain.

Impervious area alone does not always relate directly to river condition, because some impervious surfaces may be entirely disconnected from the river network. The effective imperviousness is the proportion of a catchment covered by impervious surface and connected to the stream by stormwater drainage pipes. This measure is more directly related to stream degradation than total impervious area (Walsh et al. 2005b). Recently researchers have begun to focus on effective impervious area and have found it better explains water quality variations than does total impervious surface area (Hatt et al. 2004). For future advances in our understanding of urban land-use effects on streams, hydrologic metrics will need to be developed that quantify how connected impervious area is to the stream network (Booth et al. 2004). In our proposed research we will aim to evaluate the use of stable isotope metrics to help quantify cumulative effects of upstream effective impervious area in the Coastal Plain of North Carolina.

Past research has shown that increased imperviousness in various North Carolina settings has resulted in increased peak stormflows (Mason, Jr. et al. 2001) and increased variability of streamflow and stage (McMahon et al. 2003). A growing body of work shows that physical, chemical, and biological degradation of water quality and stream channel alteration has occurred throughout North Carolina as a result of increased impervious area and stormwater stresses (Lenat and Crawford 1994, Line et al. 1997, Mallin et al. 2001, Borden et al. 2002, Line et al. 2002, Gage et al. 2004, Carle et al. 2005, Ulseth and Hershey 2005). However, there have been few documented studies on the effects of urban land-use and impervious area on baseflow contributions to streams in any of the physiographic regions of North Carolina.

Streams in the Coastal Plain physiographic province are important because they nourish bottomland hardwood forest systems, provide habitat for fish and wildlife, feed coastal and estuarine systems, provide water for domestic and industrial uses, and provide sites for wastewater disposal. Low-order Coastal Plain streams are typically blackwater streams because of their gentle gradients, long residence times, and associated dissolution of abundant organic matter within and adjacent to these rivers (Hupp 2000). Coastal Plain watersheds in North Carolina typically lose large amounts of water to evapotranspiration, particularly during summer months. This results in an extreme decrease in baseflows during the summer and the potential for the cessation of flows. A comparison of Coastal Plain and mountain hydrology in North Carolina revealed that annual evapotranspiration in a Coastal Plain watershed in Carteret County was 70% of the precipitation input, whereas the mountain site at Coweeta returned 47% of its precipitation by evapotranspiration (Sun et al. 2002). These differences are due to warmer climate in the Coastal Plain and gentler topography. Flooding recurrence of Coastal Plain streams differs markedly from other physiographic regions. Bankfull recurrence intervals in North Carolina Coastal Plain streams have been found to be much shorter than in other settings, a recent study indicates that bankfull recurrence for Coastal Plain streams may be on the order of 0.19 years (Sweet and Geratz 2003). In other settings bankfull recurrence interval is typically estimated at 1.5 years (Brooks et al 2003). This indicates that Coastal Plain streams are much more likely to overtop their banks in a given year than streams in other topographic settings.

River-ground water interactions in the Coastal Plain are controlled by the near-channel stratigraphic framework and the surficial aquifer. The surficial aquifer that extends across the Coastal Plain of North Carolina ranges from 4-224 feet thick (Lautier 2001). It consists of fine grained sand, silt, clay, and shell materials typically of Holocene to Pleistocene in age. The surficial aquifer is the likely source of a significant portion of annual stream flow in Coastal Plain rivers. The complex stratigraphy of floodplain settings, active channel sediments, and the surficial aquifer influence the direction and magnitude of ground water flows to rivers within the Coastal Plain. Age dates are rare for ground water within the surficial aquifer but recent work at the Lizzie site (Greene County, NC) showed ground waters in the surficial aquifer were less than 50 years old (n=10) and within the alluvial aquifer adjacent to tributary streams the ground waters were typically less than 10 years old (n=4) (Spruill et al. 2005). Residence times for three tributary streams at the research site were all found to be less than 30 years old. This work indicates that ground water feeding Coastal Plain streams from the surficial aquifer has a high likelihood of being affected by land-use changes that have occurred over the past 50 years. Alterations to the land cover within the Coastal Plain are likely to affect recharge to the surficial aquifer and aquifer-fed streams over relatively short timeframes; alterations in recharge may result in measurable baseflow reductions in Coastal Plain streams.

Several methods exist to evaluate baseflow contributions to streams. These include seepage runs (Zelwegger et al. 1989), physical hydrograph separation (Lim et al. 2005), stable isotope and radioactive tracing (Clark and Fritz 1997), geochemical tracers (Katz et al. 1997), piezometry (Lee and Cherry 1978), thermal monitoring (Silliman et al. 1995, O’Driscoll 2006), etc. Stable isotope tracers have not been documented to evaluate baseflow contributions to streams in the Coastal Plain of North Carolina. Precipitation isotopic data collected at Wilmington, North Carolina indicate that there is significant seasonal and storm variability in ^{18}O composition of rainfall in the region (Willey et al. 2000). Ground water isotopic composition data collected in the Coastal Plain of North Carolina is quite different from that of rainfall, therefore stable isotopes may be useful tracers of surface water-ground water interactions in this setting (Hedges 2002).

In the past stable isotope tracers have been effectively used to provide insight into hydrological processes and runoff generation from forested catchments (Buttle 1994, Kendall and McDonnell 1998). Recently stable isotope techniques have been used to quantify differences in hydrology across different physiographic settings (O’Driscoll et al. 2005) and to evaluate urban land-use alterations to hydrological processes (Sidle and Lee 1999, Burns et al. 2005). There is great potential to advance the understanding of land-use effects on hydrology through the application of stable isotope techniques (Burns 2002). In this study we aim to document the applicability of stable isotope techniques to reflect land-use alterations to hydrology in the Coastal Plain. There is promise that this approach can be used to evaluate watershed-scale stormwater runoff effects in this and other physiographic settings.

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(7) **Training Potential**

Students	**Field of specialty**	**Degrees expected**
(1) Graduate	Geology	M.S.
(1) Undergraduate	Geology	B.S.

One graduate student and one undergraduate student in the field of geology will participate in field data collection, data compilation and analyses, and data interpretation as a result of this proposed project. One M.S. thesis is expected to document this study. The urban streams within the monitoring network are typically located less than 5-miles from ECU campus; these sites will also be used for numerous class field trips for hydrology-related courses.

(8) **Investigators' Qualifications: (next four pages)**

Michael A. O'Driscoll
East Carolina University, Department of Geology, 204 Graham Building, Greenville, NC 27858
Ph: 252.328.5578 • odriscollm@mail.ecu.edu

Professional Preparation

University of Connecticut	Geology	B.S. (Cum Laude), Dec. 1996
The Pennsylvania State University	Environmental Pollution Control	Masters, May 1999
The Pennsylvania State University	Geosciences	M.S., May 2000
The Pennsylvania State University	Forest Hydrology	Ph.D., Dec. 2004

Appointments

East Carolina University	Assistant Professor-Geology Department	Spring 2005
East Carolina University	Instructor-Geology Department	Fall 2004
The Pennsylvania State University	Adjunct Faculty-Geology (Harrisburg)	Fall 2003
The Pennsylvania State University	Research Assistant	Jan. 1999-Aug. 2003
The Pennsylvania State University	Teaching Assistant-Hydrogeology	Fall 1998
The Pennsylvania State University	Lab Instructor-Physical Geology	Fall 1997- Spring 1998
Hygenix, Inc. - Stamford, CT	Environmental Consultant	Dec. 1996 – Aug. 1997
United States Navy - Norfolk, VA	Aviation Mechanic, Petty Officer 3rd Class	1990–1994

Publications

O'Driscoll, M.A. and DeWalle, D.R. Stream-air temperature relations to classify stream-ground water interactions in a karst setting, central Pennsylvania , USA . 2006. Journal of Hydrology 329:140-153.

O'Driscoll, M.A., S.R. Riggs, D.V. Ames, M. M. Brinson, D.R. Corbett, and D. J. Mallinson. 2006. Geomorphic, Ecologic, and Hydrologic Dynamics of Merchants Millpond, North Carolina . In "Hydrology and Management of Forested Wetlands." Proceedings of The ASABE International Conference on Hydrology and Management of Forested Wetlands. New Bern , North Carolina, USA. Pp. 423-431.

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O’Driscoll, M.A. and DeWalle, D.R. 2002. Episodic stream/aquifer interactions in a Pennsylvania urban/agricultural watershed. In: Ground Water/Surface Water Interactions. Kenny, J.F. (ed.) AWRA 2002 Summer Specialty Conference Proceedings, American Water Resources Association, Middleburg, Virginia. pp. 535-540.

Professional Activities

- **American Water Resources Association -** Hydrology and Watershed Management Committee. Journal of the American Water Resources Association - Manuscript Reviewer.
- **Geological Society of America (GSA)** - South East Section GSA Grant Reviewer.
- **National Science Foundation -** Grant reviewer for Hydrological Sciences Section.
- **Otter Creek Natural Area Committee** (2004-present).
- **American Geophysical Union, North Carolina Water Resources Association, National Ground Water Association, International Association of Hydrological Sciences**

Funded Grants

Zarate, M., O’Driscoll, M., and Humphrey, C. 2006. The Effects of On-Site Wastewater Systems on Shallow Ground-Water Quality in Coastal North Carolina. ECU Division of Research and Graduate Studies 2006 Research Development Grant Program. $25,000.

O’Driscoll, M. A. 2006-2007. The Influence of Streamflow and Storm Runoff on Nutrient Transport to an Estuary. ECU, Thomas Harriot College of Arts and Sciences Research Award. $4,500.

Brinson, M.M., Rheinhardt, R.D., Christian, R.R., and O’Driscoll, M.A. 2006-2008. Development and Training for Reach and Watershed Assessment Protocols in Coastal Plain North Carolina. North Carolina Department of Environment and Natural Resources, Ecosystem Enhancement Program. $218, 555.

O’Driscoll, M.A. and Mallinson, D.J. 2006. Characterization of surface water/ground water interactions along the Tar River using ground penetrating radar. North Carolina Water Resources Research Institute 2005-2006 Program. $45,129.

O’Driscoll, M.A. 2006. Land-use effects on river-ground water interactions in the Coastal Plain of North Carolina. East Carolina University Research/Creative Activity Grants 2005-2006. $18,331.

O’Driscoll, M.A. 2005. An outdoor field laboratory for hydrology at Otter Creek Natural Area and the Voice of America site. East Carolina University Student Computer and Technology Fee Innovative Project Proposal, 2004-2005. $13,973.

O’Driscoll, M.A. and DeWalle, D.R. 2003. Stream-ground water interactions in a carbonate watershed. College of Agricultural Science Competitive Grant Program for Graduate Students, Penn State University. $2,000.

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MARK M. BRINSON
Department of Biology
East Carolina University
Greenville, North Carolina 27858
Office: (252) 328-6307

Home: 209 Pineview Drive
Greenville, NC 27834
(252) 756-2658
brinsonm@mail.ecu.edu

Education
B.S. 1965, Heidelberg College, Tiffin, Ohio; M.S. 1967, University of Michigan, Ann Arbor, Michigan; Ph.D. 1973, University of Florida, Gainesville, Florida

National Committees Testimony before Subcommittee on Environmental Protection of the Committee on Environment and Public Works, U.S. Senate, April 9, 1991. (S. Hrg. 102-69); Testimony before Committee on Interior and Insular Affairs, U.S. House of Representatives, February 26, 1992; Panel of USDA Competitive Grants Program (July 1991); Site reviews of 2 NSF-LTER projects (1991); Committee on Wetlands Characterization, National Research Council, National Academy of Sciences (1993-1995); Member of Commission on Ecosystem Management, IUCN (The World Conservation Union) 1998-2000; American Institute of Biological Science, Board of Directors, Chair of Public Policy Review Committee (1998-2000): Chair of Committee on Riparian Areas: Functions and Strategies for Management, National Research Council, National Academy of Sciences (2000-2002).

Professional Experience Department of Biology, East Carolina University, Assistant Professor, 1973-77; Associate Professor, 1977-81; Professor, 1981-present; Director of Graduate Studies in Biology, 1981-86. Ecologist, Office of Biological Services, U.S. Fish and Wildlife Service, 1979-80.
Visiting Assistant Professor of Botany, University of North Carolina at Chapel Hill, Summer 1976.
Research Associate, Center for Aquatic Sciences, University of Florida, Summer 1971.
Fisheries Biologist, Peace Corps, Turrialba, Costa Rica, 1967-69.
Honors Foreign Area Fellowship Program (Ford Foundation) for research in Latin America (1971-73); Award for Excellence for Graduate Research, Ph.D.--Institute of Food and Agricultural Sciences, University of Florida (1974); Helms Faculty Research Award from Sigma Xi, East Carolina University (1978 and 1988) American Men and Women of Science (1976); National Wetlands Award for Science Research cosponsored by the Environmental Law Institute and the Environmental Protection Agency (1995); East Carolina University, College of Arts and Sciences, Distinguished Professor Award (1997); East Carolina University, Board of Trustees, Lifetime Achievement Award (1997); East Carolina University, Distinguished Research Professor Award (1997); Merit Award, Society of Wetland Scientists (1998).

Selected Refereed Journal Publications (since 1993)
Brinson, M.M. 1993. Gradients in the functioning of wetlands along environmental gradients. Wetlands 13:65-74.
Moorhead, K.K. and M.M. Brinson. 1995. Response of wetlands to rising sea level in the lower Coastal Plain of North Carolina. Ecological Applications 5:261-271.
Young, D.R., G. Shao, and M.M. Brinson. 1995. The impact of the October 1991 northeaster storm on barrier island shrub thickets (*Myrica cerifera*). Journal of Coastal Research 11:1322-1328.
Brinson, M.M., R.R. Christian, and L.K. Blum. 1995. Multiple states in the sea-level induced transition from terrestrial forest to estuary. Estuaries 18:648-659.
Brinson, M. M. and R. Rheinhardt. 1996. The role of reference wetlands in functional assessment and mitigation. Ecological Applications 6:69-76.
Rheinhardt, R.D., M.M. Brinson, and P.M. Farley. 1997. Applying wetland reference data to functional assessment, mitigation, and restoration. Wetlands 17:195-215.

Michener, W.K., E.R. Blood, K. L. Bildstein, Mark M. Brinson, and L.R. Gardner. 1997. Climate change, hurricanes and tropical storms, and rising sea level in coastal wetlands. Ecological Applications 7(3):770-801.

Brinson, M.M. and R.R. Christian. 1999. Stability of *Juncus roemerianus* patches in a salt marsh. Wetlands 1:171-191.

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Whigham, D.F., L.C. Lee, M. M. Brinson, R. D. Rheinhardt, M.C. Rains, J.A. Mason, H. Kahn, M.B. Ruhlman, and W.L. Nutter. 1999. Hydrogeomorphic (HGM) assessment – a test of user consistency. Wetlands 19:560-569.

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Kroes, D. and M. M. Brinson. 2004. Occurrence of riverine wetlands on floodplains along a climatic gradient. Wetlands 24:167-177.

Refereed and Invited Chapters (since 1998)

Brinson, M.M. and R. D. Rheinhardt. 1998. Wetland functions and relations to societal values. Chapter 2 (pp. 29-48) In M. Messina and W. Conner (editors). Southern Forested Wetlands: Ecology and Management, Lewis Publishers, Ann Arbor, Michigan.

Brinson, M.M., R.D. Smith, D.F. Whigham, L.C. Lee, R.D. Rheinhardt, and W.L. Nutter. 1998. Progress in development of the hydrogeomorphic approach for assessing the functioning of wetlands. Pages 393-406, In A. J. McComb and J. A. Davis, editors. Wetlands for the Future. Gleneagles Publishing, Adelaide, Australia.

Brinson, M.M. and J. Verhoeven 1999. Chapter 8. Riparian Forests. Pages 265-299 *in* M.L. Hunter (editor). Maintaining Biodiversity in Forested Ecosystems. Cambridge University Press, Cambridge, England.

Richardson, J.L. and M.M. Brinson. 2000. Chapter 9. Wetland soils and the hydrogeomorphic classification of wetlands. Pages 209-227 in J.L. Richardson and M.J. Vepraskas (editors). Wetland Soils: Genesis, Hydrology, Landscapes, and Classification. Lewis Publishers, Boca Raton, Florida, USA.

Christian, R.R., L.E. Stasavich, C.R. Thomas, and M.M. Brinson. 2000. Reference is a moving target in sea-level controlled wetlands. Pages 805-825. In M.P. Weinstein and D.A. Kreeger (editors). Concepts and Controversies in Tidal Marsh Ecology. Kluwer Press, The Netherlands.

Poff, N.L., M.M. Brinson, and J.W. Day, Jr. 2002. Potential Impacts on Inland Freshwater and Coastal Wetland Ecosystems in the United States. Pew Center on Global Climate Change, Arlington, VA. 45 pp. (special report)

Lugo, A.E., M.M. Brinson, and S. Brown (eds.). 1989. Forested Wetlands. Vol. 15 of Ecosystems of the World Series. Elsevier Scientific Publishers, Amsterdam. 527 pp.

Antibiotic Resistance and Water Quality: Land Application of Swine Lagoon Effluent as a Potential Source of Antibiotic Resistant Genes in Surface Water

Basic Information

Title:	Antibiotic Resistance and Water Quality: Land Application of Swine Lagoon Effluent as a Potential Source of Antibiotic Resistant Genes in Surface Water
Project Number:	2007NC76B
Start Date:	3/1/2007
End Date:	2/28/2009
Funding Source:	104B
Congressional District:	2
Research Category:	Water Quality
Focus Category:	Water Quality, Agriculture, None
Descriptors:	
Principal Investigators:	Alexandria Graves, Daniel Israel

Publication

Title

Antibiotic Resistance and Water Quality: Land Application of Swine Lagoon Effluent as a Potential Source of Antibiotic Resistant Genes in Surface Water

Project Summary

The use of antibiotics in animals is suspected to be a major route of the transference of antibiotic resistant bacteria to humans, even when different antibiotics are used in animals than in people. Mathematical models have been used to evaluate the medical impacts of simultaneously using the same antibiotic in food animals and human medicine. Analysis from the mathematical models demonstrates that animal antibiotic use may hasten the appearance of antibiotic resistance and decrease the efficacy of antibiotic used in humans. A number or reports have specifically linked antibiotic use in livestock with the spread of antibiotic resistant pathogenic bacterial to humans. North Carolina is the home of our Nation’s second largest swine industry. Most of this swine production is restricted to a small geographical area in southeastern North Carolina. This high concentration of swine production may increase the risk of antibiotic resistant bacteria from swine operations reaching the nearby surface waters. If antibiotic resistance and the presence of antibiotic resistant genes are occurring at an elevated level in swine waste, then it logically follows that antibiotic resistant genes found in bacteria are potentially discharged during land application of swine lagoon effluent and have the potential to reach nearby surface waters. The goal of this study is to evaluate the association of antibiotic resistance genes found in *E.coli* isolated from swine with the actual phenotypic expression of the resistance. Additionally to develop an antibiotic resistance database for *E. coli* isolates from a commercial swine facility and assess its efficacy for tracking movement of bacteria from swine confinement houses to surface waters. The appearance of swine-manure derived bacteria in shallow groundwater near the stream or in the stream would document the need for improved mitigation strategies. To establish that swine manure-derived bacteria are discharged to surface waters, source tracking methods will be used.

The predominant manure management choice for swine is the lagoon system. Anaerobic lagoons are widely used in temperate climates in the United States for the treatment of swine manure. They are simple to manage and very effective in reducing organic matter and nutrients when properly designed and operated (Bicudo et al, 1999). Anaerobic lagoons store, treat and minimally dilute the waste from concentrated animal feeding operations (CAFO). Lagoons, however, were not designed to control pathogens, despite the fact that swine manure contains as high as a billion protozoa, fungi and bacteria per gram.

Previous studies showed that pathogens can persist in swine lagoon liquid and sludge, in manure piles, and in waste litter (Plym-Forshell 1995; Radtke and Gist 1989). Pathogens are more likely to persist in liquid or moist waste, and in sludge or lagoon treatments, which do not heat manure to a high enough temperature to kill pathogens (Kudva et al. 1998). Hog manure may contain pathogens like *Cryptosporidium* and *Salmonella*, which can cause diarrhea in normal healthy adults, but can be fatal in children, the elderly and other groups at risk. (Sobsey et al, 1999). Raw hog waste applied to crops can contain 100 to 10,000 times the number of pathogens that is allowed in treated human waste (Sobsey et al, 1999). However, since raw hog waste is rarely if ever applied to crop land in North Carolina, the level of human pathogens in effluent from treatment lagoons applied to crops is likely to be lower than that reported for raw manure

(Sobsey, et al. 1999) Nevertheless, since pathogens move easily through air and water, there is potential for transmission from swine operations to humans.

The goal of this study is to evaluate the association of antibiotic resistance genes found in *E.coli* isolated from swine with the actual phenotypic expression of the resistance. Additionally to develop an antibiotic resistance database for *E. coli* isolates from a commercial swine facility and assess its efficacy for tracking movement of bacteria from swine confinement houses to surface waters.

1. Determine the relationship between presence of antibiotic resistance genes for tetracycline, sulfonamides, streptomycin and apramycin resistant genes found in *E. coli* strains from swine manure, lagoon effluent and nearby ground and surface waters with the actual phenotypic expression of the resistance.
2. Develop a database of antibiotic resistance patterns for *E. coli* isolated from swine manure, cattle manure, wildlife manure, human and pets.
3. Evaluate the usefulness of this database for assessing movement (or dispersal) of *E. coli* from a confined swine operation to a nearby stream.

Methodology

The Soil Science Department has well equipped laboratories for molecular and microbiological analysis of manure, water/wastewater, and soil. Dr. Graves's laboratory is equipped with a Mastercycler ep *realplex* real-time thermal cycling system, eppendorf thermocycler for conventional PCR, agarose gel electrophoresis units, gel documentation systems, membrane –filter manifolds, centrifuges, water baths, incubators, refrigerators, -20°C and -80°C freezers. The lab also houses PC computers with internet access.

Study Site: The study site (Figure 1) is a commercial swine farm with a standing herd of 4400 finishing animals, located in a 275 ha watershed along the upper reach of Six Runs Creek, which flows in a southerly direction in eastern Sampson County, NC. The study site is approximately 18 km north of Clinton, NC. The study site has two waste application fields. The stream adjacent to waste application field 1 flows in a channel, but the segment adjacent to waste application field 2 is impounded by two beaver dams and forms an elongated pond. Below the lower beaver dam the stream flows in a channel as it exits the producer's property. Four swine operations with 23 swine houses are located in this watershed. Fields receiving swine-lagoon effluent (approximately 40 ha) and cropped with coastal bermuda grass managed for hay or as grazed pastures are situated on both sides of the creek. A forested riparian buffer of variable width (41 to 87m) is located between the waste application fields and the creek. Three transects of piezometers (wells) have been installed in each of two waste application fields and the adjacent forested riparian system on the west side of Six Runs Creek for sampling of shallow ground water. Each transect has four or five well nests positioned on the side slope of the field, at the field edge, in the riparian zone, and at the stream edge. In the waste application fields, wells within a nest have been placed 1 m apart and screened at three different depths: near top of water table, and at two greater depths below the water table (Israel et al., 2005).

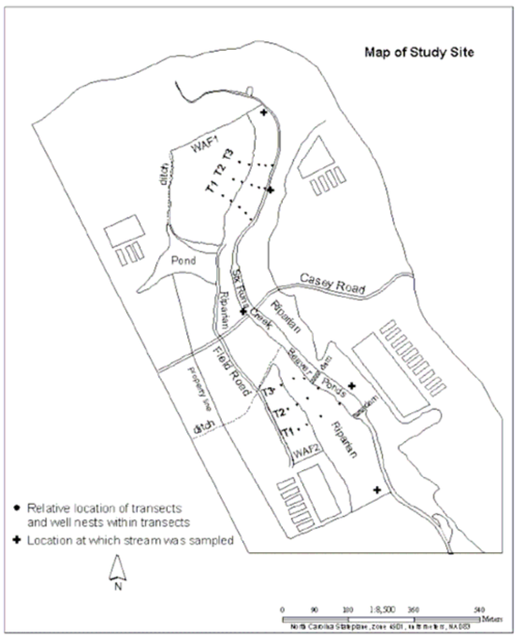

Figure 1. Map of study site. Figure found in Israel et al., 2005

Procedures for Objective 1: A combined total of 300 *E. coli* isolates from swine houses, lagoons, ground and surface waters will be evaluated for antibiotic resistance genes and phenotypic expression of antibiotic resistances. Shallow groundwater will be sampled from wells in the sprayfield and at the stream edge and the stream will be sampled upstream, adjacent to and down stream of the swine operation. Swine manure and lagoon effluent samples will be serially diluted (surface water and groundwater samples will not be diluted) and filtered on membrane filters. Filters will be transferred to plates and incubated at 44.5°C. After 24 h single colonies will be picked and transferred to liquid media and incubated at 37°C. After 24 h an aliquot of each culture will be taken for PCR analysis and another aliquot will be transferred to micro-well plates for the antibiotic resistance evaluations.

PCR detection of resistance genes. Bacterial lysates will be used as templates for the PCR reactions. Lysates will be prepared by resuspending a loopful of bacteria from a fresh overnight culture on a blood agar plate will be resuspended in 500µl of water, homogenized and heated at 95°C for 15 min. After cooling to room temperature, suspensions will be centrifuged for 3 min at maximum speed in a microcentrifuge. A 1-µl volume of the supernatant will be used as a template for each 25-µl PCR mixture. The primers and protocols for major resistance genes for tetracycline (*tetA*, *tetB*, and *tetC*), sulfonamides (*sul1*, *sul2*, and *sul3*), streptomycinspectinomycin (*strA*/*strB*, *aadA*), and apramycin [*aac(3)IV*)] are described in Table 1. All polymerase chain reactions will be completed with the following temperature cycling: 1 cycle of 4 min at 95°C; 35 cycles, each consisting of 1 min at 95°C, 1 min at annealing temperature given in Table 1 followed by 1 min at 72°C; and 1 cycle of 7 min at 72°C. SYBR Green I (Applied Biosystems) will be used to detect the amplified product. The product will be run through gel electrophoresis to confirm fragment location.

TABLE 1. Single PCR conditions and control strains

Gene	Primer name	Primer sequence	Anneal (°C)	Fragment size (bp)	Positive control
aadA	4F[a]	GTGGATGGCGGCCTGAAGCC	68	525	AMR-002[d]
	4R[a]	AATGCCCAGTCGGCAGCG			
strA	2F[a]	CCTGGTGATAACGGCAATTC	55	546	AMR-009[d]
	2R[a]	CCAATCGCAGATAGAAGGC			
strB	3F[a]	ATCGTCAAGGGATTGAAACC	55	509	AMR-009[d]
	3R[a]	GGATCGTAGAACATATTGGC			
tetA	TetA-L[b]	GGCGGTCTTCTTCATCATGC	64	502	RO8[d]
	TetA-R[b]	CGGCAGGCAGAGCAAGTAGA			
tetB	TetB-L[b]	CATTAATAGGCGCATCGCTG	64	930	PB#11[d]
	TetB-R[b]	TGAAGGTCATCGATAGCAGG			
tetC	TetC-L[b]	GCTGTAGGCATAGGCTTGGT	64	888	PB#02[d]
	TetC-R[b]	GCCGGAAGCGAGAAGAATCA			
sul1	Sul1-L[b]	GTGACGGTGTTCGGCATTCT	68	779	AMR-130[d]
	Sul1-R[b]	TCCGAGAAGGTGATTGCGCT			
sul2	Sul2-L[b]	CGGCATCGTCAACATAACCT	66	721	AMR-130[d]
	Sul2-R[b]	TGTGCGGATGAAGTCAGCTC			
sul3	Sul3-F[c]	GAGCAAGATTTTTGGAATCG	51	880	RL0044[c]
	Sul3-R[c]	CATCTGCAGCTAACCTAGGGCTTTGGA			
aac(3)IV	Aac4-L[d]	TGCTGGTCCACAGCTCCTTC	59	653	AMR-075[d]
	Aac4-R[d]	CGGATGCAGGAAGATCAA			

[a] Reference: Boerlin et al., 2005; [b] Reference: Lanz et al., 2003; [c] Reference: Perreten and Boerlin, 2003; [d] Reference: Boerlin et al., 2005.

Antibiotic resistance analysis of isolates. Various antibiotic concentrations will be used to determine antibiotic resistance patterns in target microorganisms (Table 2). The antibiotics/concentrations were selected based on previous success from other ARA studies and their common use in human and veterinary practice (Mathew et al., 1999). Each of the thirty-eight antibiotic/concentrations is added separately to flasks of autoclaved and cooled Trypticase Soy Agar (TSA, BBL) from stock antibiotic solutions to achieve the desired concentration, and then poured into sterile 15x100mm petri dishes. Control plates (no antibiotics) are included with each set. Isolates are transferred from the microwell plate using a stainless steel 48-prong replica plater (Sigma). The replicator is flame-sterilized (95% ethanol) after inoculation of each TSA plate. The inoculant is allowed to soak into the agar and the plates are then incubated for 48 hours at 37°C. Resistance to an antibiotic is determined by comparing each isolate to the growth of that isolate on the control plate. A one (1) is recorded if that isolate grew (a round colony, mostly filled) and a zero (0) is recorded for no growth. This is repeated for each isolate on each of the 30 antibiotic plates.

This information will allow correlation of occurrence of antibiotic resistance genes carried by isolates with the expression of antibiotic resistances encoded by these genes. This will allow an assessment of the level of expression of antibiotic resistance genes in the *E. coli* population.

Table 2. Antibiotics and concentrations used in ARA.

Antibiotics	**Concentrations (μg/ml)**	**No. of Variables**
Erythromycin	60, 70, 90, and 100	4
Neomycin	2.5, 5.0, and 10	3
Oxytetracycline	8, 16, 32, 64, and 128	5
Streptomycin	8, 16, 32, 64, and 128	5
Tetracycline	8, 16, 32, 64, and 128	5
Cephalothin	16, 32, 64, and 128	4
Apramycin	16, 32, and 64	3
Sulfamethoxazole	64, 128, 256, and 512	4
Trimethoprim-Sulfamethoxazole	8, 16, 32, 64, and 128	5
Control	No antibiotic	2
Total		40

Procedures for Objective 2. Strains of *E coli* will be isolated from known fecal waste samples to develop a known source library. No more than 10 isolates will be taken from a given sample of each manure source to build a database of 1000-1200 isolates. The known source categories will be composed of swine, cattle, wildlife and pets. Over 300 *E. coli* isolates from swine and 300 *E. coli* isolates from cattle have already been collected for database development. Antibiotic resistance analysis on 1000-1200 known isolates will be performed as described under Objective 1.

Statistical Analysis for ARA: Variables for the analyses include the number of antibiotics used and the degree of pooling of sources. Each analysis produces a classification set for every known source isolate. The correct classification rates are calculated using one set of antibiotic resistance patterns (ARPs) both to establish the classification rule and as test subjects (Harwood et al., 2000). The number of isolates from a given source that are placed in the correct source category by discriminant analysis is termed the rate of correct classification. The average rate of correct classification (ARCC) for the database is obtained by averaging the correct classification percentages for all sources (Harwood et al., 2000). The holdout method of cross validation, in which isolates from known sources are randomly removed from the data set and treated as test subjects, will be used as a more rigorous test of the predictive power of the databases (Harwood et al., 2000). To determine whether the known database are large enough or has ample representation, artificial clustering will be used. Artificial clustering involves randomly assigning equal numbers of isolates from each source and applying discriminant analysis to determine the random ARCC. Our database will contain 4 source types, swine, cattle, wildlife and pets. The random ARCC should be approximately 25% for each source. Thus, any percent significantly greater than the 25% ARCC indicates that the known source database is not representative. If the ARCC for a source segment of the database is found not to be representative, isolates will be added until the problem is corrected. By doing so, assures that the database will serve as a good point of reference for identifying unknown source isolates collected from Six Runs Creek. The development and validation of this database will allow determination of the source of unknown *E. coli* isolates obtained from the Six Runs Creek.

Procedures for Objective 3. Water samples will be collected from a total of 5 stream sampling sites, once a month for nine months. Sampling sites will consist of upstream (above waste application field 1, see Figure 1) and downstream sites in relation to the swine facility. The sampling regime is designed to capture possible seasonal variation in host sources contributing bacterial loading to Six Runs Creek. Ground water samples will be collected from the wells of transect two at each waste application fields (figure 1). Sampling from these sites will occur once every other month for nine months.

Isolation of *E. coli* will be performed by membrane filtration of a known volume of a water sample passed through a membrane filter that is then placed on media that is selective for the target microorganism. After incubation for 24 hr in a 44.5°C water bath, colonies will be transferred to 96-microwell plates containing 0.2 ml colilert broth specific for the target microorganism, and incubated for 24 h at 37°C. Twenty-four isolates from each water sample will be evaluated using antibiotic resistance analysis to determine its source. Antibiotic resistance analysis will be performed as described under objective 1. Isolates identified as swine will be evaluated for the presence antibiotic resistant genes using procedures described in objective 1.

Principal Findings

Over 1,600 bacterial isolates collected from water, swine feces, and lagoon samples have been evaluated by antibiotic resistance analysis. To date, preliminary data from *E. coli* monitoring of groundwater suggests that there is a very low occurrence of *E. coli* as the average MPN/100ml for the twenty water samples collected is 2.0 with a 95% lower confidence limit of 0.3 and an upper confidence limit of 7.1. Thus suggests that the waste management practices of the integrator at this swine facility are successfully minimizing the introduction of fecal bacteria into the environment. The isolates recovered from the water samples have been analyzed by antibiotic resistance and will also be evaluated for antibiotic resistant genes.

Significance

Microbial resistance to antibiotics is spreading fast; incidence of vancomycin resistance has increased from less than 1% to 17% within a span of 10 years (Pfaller et al., 1998). This study is intended to evaluate the association of antibiotic resistance genes found in *E.coli* isolated from swine with the actual phenotypic expression of the resistance. Additionally to develop an antibiotic resistance database for *E. coli* isolates from a commercial swine facility and assess its efficacy for tracking movement of bacteria from swine confinement houses to surface waters. Quantitative polymerase chain reaction will provide robust, sensitive and highly discriminant data. The results of this research will provide important information regarding the role of land application of lagoon effluent in spreading of bacteria with antibiotic resistance genes to surface waters. Early diagnosis of the problem will allow for the development of improved technologies and mitigation strategies.

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Information Transfer Program Introduction

In addition to activities related to specific research projects, WRRI maintains a strong information transfer program by cooperating with various state agencies, municipalities, and professional organizations to sponsor workshops and other events and by seeking grants for relevant activities. During the current fiscal year, WRRI continued to be designated by the N.C. Board of Examiners for Engineers and Surveyors as an Approved Sponsor of Continuing Professional Competency activity for Professional Engineers and Surveyors licensed by the State of North Carolina. In addition, WRRI is an approved sponsor for the N.C. Board of Landscape Architects to offer contact hours. This allows WRRI to offer Professional Development Hours to engineers and surveyors for attending our water resources research seminars, our Annual Conference, and other workshops we sponsor

The WRRI Information Transfer Program includes the WRRI Annual Conference, which the institute has done since 1998. The 10th Annual Conference was held on March 27−28, 2007 and was titled “Managing Public Water Supplies,” held at the NCSU McKimmon Center in Raleigh, NC. It is the state's premier water research conference. Many questions about North Carolina's water resources will be addressed through research presented by university and corporate researchers, students, local, state, and federal government agency representatives, and environmental professionals. The Institute's goal is to provide a forum for attendees to become informed and educated on the most current research addressing water resource issues in North Carolina, as well as network and discuss water−related issues with other attendees.

The WRRI Information Transfer Program includes workshops supported by the NC Department of Environment and Natural Resources (DENR), Land Quality Section along with the NC Sedimentation Control Commission (SCC). Workshops held during this period include: (1) Two Spring Erosion and Sediment Control Planning and Design Workshop, 3/9−10/2007 Wilmington, NC, 4/17−18/2007 Boone, NC; (2) Two Fall Planning and Design Erosion and Sedimentation Control Workshops, 9/25−26/2007 Greenville, NC; and 10/30−31/2007 Hickory, NC; and (3) Erosion and Sedimentation Control Local Programs Training Workshop, 1/29−30/2008 Southern Pines, NC.

Another way WRRI provides Information Transfer is through the North Carolina Water Resources Association (NCWRA) Luncheon and Forums: (1) September 10, 2007: “Using the Best Climate Information Technology for Managing Water Resources,” Dr. Ryan Boyles, State Climatologist, State Climate Office of NC; (2) December 3, 2007: ”Ecosystem Enhancement Program – Challenges Lying Ahead,” Suzanne Klimek, Director of Operations, NC Ecosystem Enhancement Program; and, (3) February 4, 2008: “Emerging Contaminants – A Water Quality Odyssey,” Mary Giorgino, Water Quality Specialist, US Geological Survey, North Carolina Water Science Center.

WRRI maintains six electronic mail lists (listservs): (1) Water Research list − 180 subscribers – inform water researchers from NC universities about calls for papers, grants, upcoming conferences, student internships, etc.; (2) WRRI−News list − 740 subscribers − informs researchers, local governments, municipalities, interest groups etc. about calls for papers, grants, upcoming conferences and events, etc.; (3) NCWRA−info list − 270 subscribers − provides information of the North Carolina Water Resources Association sponsored events; (4) Sediments list − 215 subscribers − sent out SEDIMENTS newsletter and information on erosion and sediment control regulations and educational workshops/seminars; (5) Urban Water Consortium (UWC) for Urban Water Consortium member communications; (6) and UWC−Stormwater Group list for the UWC Stormwater Group communications.

WRRI maintains its own website (http://www.ncsu.edu/wrri). The website provides on−line access to the WRRI−News, the WRRI technical report summaries, water research seminars, and information on WRRI−sponsored workshops, conferences, and seminars. During this fiscal year, WRRI has scanned its old research reports and made them into searchable pdf files. Most of these reports are available on the WRRI web site and the remainder should be available later in 2008.

WRRI administers the NC Urban Water Consortium (UWC) and meets with its members quarterly. The consortium was established in 1985 by the Institute, in cooperation with several of North Carolina's larger cities to provide a program of research and development and technology transfer on water problems that urban areas share. Through this partnership, WRRI and the State of North Carolina help individual facilities and regions solve problems related to local environmental or regulatory circumstances. Participants support the program through annual dues and enhancement funds and guide the program through representation on an advisory board, selection of research topics, participation in design of requests for proposals, and review of proposals. Currently there are 11 member cities/special districts in North Carolina that met on the following dates: March 20, 2007 – Fayetteville; June 20, 2007 – Charlotte; September 27, 2007 – Raleigh; and, December 11, 2007 – High Point.

In 1998, several members of the NC UWC partnership formed a special group to sponsor research and technology transfer on issues related to urban stormwater and management. The Urban Water Consortium (UWC) Stormwater Group is administered by WRRI. Participants support the program through annual dues and enhancement funds. They guide the program through representation on an advisory board, selection of research topics, participation in the design of requests for proposals and review of proposals. Currently there are eight members that met on the following dates: April 19, 2007 – Durham; June 14, 2007 – Fayetteville; September 19, 2007 – Wilmington; and, December 12, 2007 – Winston−Salem.

The WRRI Institute News

Basic Information

Title:	The WRRI Institute News
Project Number:	2007NC117B
Start Date:	3/1/2007
End Date:	2/28/2008
Funding Source:	104B
Congressional District:	2
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	
Principal Investigators:	Kelly A Porter

Publication

Published the *WRRI News* six times during the reporting period. The WRRI News is an 8-page newsletter that covers a wide range of water-related topics from current federal and state legislation and regulatory activities to new research findings, water-related workshops and conferences, and reviews of water-related publications. The WRRI News is now sent electronically to 788 federal and state agencies, university personnel, multi-county planning regions, city and local officials, environmental groups, consultants, businesses and individuals. It is also posted on the website.

WRRI Annual Conference

Basic Information

Title:	WRRI Annual Conference
Project Number:	2007NC118B
Start Date:	3/27/2007
End Date:	3/28/2007
Funding Source:	104B
Congressional District:	2
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	
Principal Investigators:	Kelly A Porter

Publication

Convened the Annual Conference: Managing Public Water Supplies. Dr. Phil Singer, Director, UNC Drinking Water Research Center & Professor, UNC Department of Environmental Sciences & Engineering, delivered plenary addresses. Investigators from universities, agencies, industry, and consulting firms presented results of work on topics ranging from erosion and sedimentation control technologies to air borne water pollutants. Some 338 people participated in the conference. Participants had 36 technical presentations in 9 concurrent sessions from which to choose, as well as 33 technical posters to view. Abstracts were made available on the WRRI website.

WRRI Research Reports

Basic Information

Title:	WRRI Research Reports
Project Number:	2007NC119B
Start Date:	3/1/2007
End Date:	2/28/2008
Funding Source:	104B
Congressional District:	2
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	
Principal Investigators:	L. Upton Hatch

Publication

New WRRI Research Reports – During the year, the Institute published the following reports for distribution to users throughout the state and nation. Reports are now distributed through the WRRI website. The documents are uploaded into the library repository and available for downloading or printing.

WRRI-361 – Dentification and Sediment-Water Nutrient Exchange in the Upper Neuse River, by S. Whalen, University of North Carolina at Chapel Hill.

WRRI-377 – Influence of Phosphorus on the Moblization and Attenuation of lonogenic Herbicides in NC Piedmont Soils: Implications for Water Quality, by S. Vasudevan, Duke University.

WRRI-378 – Integration of High-Resolution Satellite Imagery in Cost-Effective Assessment of Land Use Practices Influencing Erosion and Sediment Yield, by S. Khorram, NC State University.

WRRI-380 – Hog Waste Treatment to Control Microbial Contamination, by M. Sobsey, University of North Carolina at Chapel Hill.

WRRI-381 – The Neuse River Basin Agricultural Nitrogen Reduction Strategy: A Programmatic Analysis, by D. Moreau, University of North Carolina at Chapel Hill.

WRRI-382 – Endocrine and Reproductive Effects of the Pharmaceutical Fluoxetine on Native Freshwater Mussels: Proximity to Measured Environmental Concentrations, by G. Cope, NC State University.

WRRI-383 – Effects of Tidal Flow on Riparian Zone Hyddraulics and Nitrogen Dynamics: Implications for Nutrient Management in Coastal Creek, by M. Piehler, University of North Carolina at Chapel Hill.

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 NCGP Award**	**NIWR−USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	4	0	2	0	6
Masters	4	0	1	0	5
Ph.D.	2	0	1	0	3
Post−Doc.	0	0	0	0	0
Total	10	0	4	0	14

Notable Awards and Achievements

Naresh Devineni, PhD student, working with Dr. Sankar Arumugam in Civil and Environmental Engineering at NC State University, received an outstanding student paper award for his presentation “Multimodel Ensembles of Streamflow Forecasts: Role of Predictor State in Developing Optimal Combinations” at the 2007 Fall AGU (American Geophysical Union) conference in San Francisco.

The presentation is from his Master's research, supported by from this project, focusing on developing climate information based streamflow forecasts for the Falls Lake.

Publications from Prior Years

1. 2005NC47B ("Characterization of Surface Water/Ground Water Interactions along the Tar River using Ground Penetrating Radar") − Conference Proceedings − O'Driscoll, M.A., D.J. Mallinson, P.K. Johnson, 2007, Geologic controls on river/ground water interactions along a Coastal Plain river, NC Water Resources Research Institute Annual Conference, "Managing Public Water Supplies," Raleigh, NC, March 27−28, 2007.
2. 2005NC49B ("A Comparison of Drought Tolerance in Common Herbaceous Wetland Macrophytes as Indicated by Plant Growth, Water Status, and Oxidative Stress") − Articles in Refereed Scientific Journals − Touchette, Brant W., Laura Iannacone, Gwendolyn Turner, and Adam Frank, 2007,"Drought Tolerance versus Drought Avoidance: A Comparison of Plant−Water Relations in Herbaceous Wetland Plants Subjected to Water Withdrawal and Repletion," WETLANDS, Vol. 27, No. 3, September 2007, pp. 656–667.